EVALUATING THE EFFECT OF HOLDING TIME AND STORAGE TEMPERATURE ON

THE CHEMICAL STABILITY OF DRINKING WATER SAMPLES COLLECTED FROM

MILITARY FORWARD DEPLOYED ENVIRONMENTS

by

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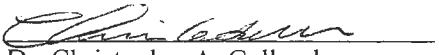
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DEDICATION

The past two years have come and gone and soon we will be on to our next assignment. A lot has happened over the course of these past two years and we have all grown together. Each of you has sacrificed so much to see me further my military career and I could not be more thankful. This accomplishment is not my accomplishment alone, it is our accomplishment. Each of you has played a vital role in us arriving at this point. It is because of the strength of character each of you possesses; we have achieved our goal and share in the success. To my wife who continues to sacrifice her professional career to ensure my success, words cannot express the gratitude and love I have for you because of what you do for me and our family. Thank you for being so supportive of my late nights and last minute trips to Aberdeen Proving Ground. To my children, thank you blessing my life with so much joy and happiness. This degree is an accomplishment I am so proud to share with each of you.

Love,

Theodore A. Wilson

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Theodore A. Wilson

ABSTRACT

EVALUATING THE EFFECT OF HOLDING TIME AND STORAGE TEMPERATURE ON THE CHEMICAL STABILITY OF DRINKING WATER SAMPLES COLLECTED FROM MILITARY FORWARD DEPLOYED ENVIRONMENTS:

Theodore A. Wilson, MSPH, 2013

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The US Army Public Health Command-Army Institute of Public Health uses Environmental Protection Agency (EPA) approved drinking water methods, or in-house developed and validated screening methods, when analyzing water samples from forward deployed environments. Each method specifies pre-analytical Maximum Holding Times (MHT) and storage temperatures for samples. The analytes in this study have MHTs as low as seven days and as high as 180 days and all require a storage temperature of 4°C. It is very challenging for US military forces to meet these guidelines when shipping samples from remote locations to the US for laboratory analyses. This study investigated the chemical stability of treated water samples when exceeding the EPA MHT and storage temperature. The compounds studied included select volatile and semi-volatile organic compounds (VOC and SVOC), Endothall, Diquat, Paraquat, and Cyanide. Three metals (Antimony, Arsenic, and Mercury) were analyzed to confirm the EPA recommendation. Contaminant-spiked water samples were stored at 4°C,

25°C, and 39°C and analyzed at pre-determined intervals over 42 days to assess chemical degradation.

The data from this study indicated that the MHT for the herbicides and some of the select VOCs and SVOC are stable beyond the current MHTs at each temperature. Chemical stability varied for individual compounds when exceeding either the MHT, storage temperature, or both. The metals were all stable throughout the duration of the study. The Cyanide stability was inconclusive due to matrix interferences. This research may lead to changes in military policy and be used as a foundational study for other temperature effects, such as a temperature profile, on stability.

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Chapter 1 - Introduction

BACKGROUND

Sustaining the fighting strength of US forces continues to be of the utmost importance to US military leaders. Maintaining the health of the fighting forces can provide a strategic advantage in combat. In every US conflict the prevention of disease and non-battle injuries (DNBI) has been a challenge. In the early 1900's influenza prevention was a major concern; good personal hygiene was implemented to prevent the spread of the disease (6). DNBI prevention continues to be a challenge as US forces deploy to areas of operation that have endemic diseases. But the world has become more industrialized and new health threats have emerged from toxic industrial chemicals and materials. Since 1991 the US military has deployed Service Members in various overseas contingency operations from armed combat to peacekeeping missions. During each of these operations, US Service Members have been at risk from environmental health threats. To manage these threats, the Joint Chiefs of Staff (JCS) developed a Force Health Protection (FHP) strategy to protect the health of Service Members while deployed. Occupational and Environmental Health (OEH) surveillance is one aspect of supporting the FHP strategy (16). OEH Surveillance supports the FHP strategy through environmental monitoring by identifying and documenting OEH hazards, providing risk assessments and making recommendations to mitigate those risks, and conducting research to continuously improve protective and preventive measures (16).

Overseas contingency operations represent a particular challenge for the Department of Defense (DoD) when analyzing OEH samples. Samples collected in

overseas theaters of operation are shipped to a DoD analytical lab in either the United States or Europe. The US Army Public Health Command (USAPHC), Army Institute of Public Health (AIPH) laboratory, located in Aberdeen Proving Ground, MD, is the primary lab that routinely receives water samples from deployed preventive medicine personnel in overseas contingency operations and analyzes the samples to determine if they contain harmful chemicals of health concern. The type and concentration level of harmful chemicals are identified from sample results then documented as potential OEH exposures to Service Members.

The US Environmental Protection Agency (EPA) has established Maximum Holding Times (MHT) and storage temperatures for environmental samples and published these guidelines in the Code of Federal Regulations Title 40 Part 141 (40 CFR 141) (8). Many analytes, such as volatile organic compounds (VOC) are temperature sensitive and are more volatile than other analytes. According to the American Society for Testing and Materials (ASTM) D4841-88 (2), The Maximum Holding Time (MHT) is defined as the maximum period of time a properly preserved sample can be stored before the degradation of the constituent of interest or change in sample matrix occurs that the systematic error exceeds the 99% confidence interval (not to exceed 15%) of the test calculated around the mean concentration found at zero time. The EPA methods provide the standards that need to be met for each individual method of analysis and the ASTM provides a method to estimate the MHT.

The EPA provides methods of analysis specific for each chemical or class of chemicals and each method is recognized as the 'gold standard' guideline for water sample acceptability. The AIPH uses a modified version of some of the EPA methods to

meet unique military operations as they relate to OEH sampling and handling conducted by US military deployed personnel. The modifications are made to address a lower collection volume or to reduce the time for analysis. While unique military operations may prevent sample collectors from meeting established EPA requirements for sample handling as outlined in each method of analysis, USAPHC's modified methods are intended to be equivalent to the standard methods thus ensuring quality sample collection and analysis.

Previous work in Afghanistan by Gellasch and Calix (12) underscored the difficulty in shipping environmental samples with short hold times and low temperature storage requirements from remote operational locations to a military analytical laboratory within the recommended EPA Maximum Holding Time (MHT) and storage temperature guidelines. Table 1 lists the holding times and temperature storage requirements for the analytes being studied in this research. It has been historically difficult to meet these guidelines for samples collected at Forward Operating Bases (FOB). These samples must be transported to an air base in theater before shipment to the analytical lab. The shortest hold time is immediate for pH, which is often analyzed in the field. The hold time for other analytes range from 48 hours to 180 days.

In addition to the challenge of meeting the EPA MHT's and storage temperature requirements, there has been concern over the ability to detect cyanide (CN) in drinking water samples due to the many interfering constituents associated with CN analysis. Carbonates, aldehydes, and oxidants such as chlorine are known interferences for CN recovery from water, according to the OIA1677 method of analysis (3). US military forces primarily use chlorine for drinking water disinfection due to its low cost, ease of

use, availability, and residual disinfection. Other common interferences are sulfides, glucose and other sugars, fatty acids, thiocyanate, and other sulfur containing compounds (4). OIA 1677 suggests using ascorbic acid to reduce the chlorine in samples known or suspected to contain chlorine, however, USAPHC uses sodium hydroxide (NaOH) as the preservative in CN collection vials based on ASTM D7365 which states samples must be analyzed within 24 hours to avoid CN degradation when using ascorbic acid (3).

Analyte	EPA MHT (Days)	EPA Storage Temp (°C)	Method of Analysis
Endothall	7	4	EPA 548.1
Diquat / Paraquat	7	4	DLS 519 (LC/MS)
VOC	14	4	EPA 524.2
SVOC	14	4	EPA 525.2 Modified
Cyanide	14	4	OIA 1677
Mercury	28	Not Specified	EPA 200.8
Antimony & Arsenic	180	Not Specified	EPA 200.8

*DLS – Directorate Laboratory Sciences; OIA – OI Analytical

Table 1: EPA MHT, Storage Temperature, and method of analysis for selected constituents.

The primary method of deployment environmental sample cold chain management is accomplished by using pre-frozen gel packs. Summer temperatures in deployed areas such as Iraq and Afghanistan often reach temperatures above 45°C and are a challenge to overcome as the pre-frozen gel packs used for shipping samples are often not sufficient to maintain the 4°C temperature requirement for storage and shipment for the duration of the shipment interval from point of collection to the analytical laboratory. During the summer months the receipt temperatures are the highest. The

most recent data set available for this study contained 224 treated water sampling kits from various locations in Afghanistan that were involved in Operation Enduring Freedom (OEF) and received between 21 January 2011 and 28 December 2011, hereafter to be referred to as the 2011 data set. Figure 1 illustrates the receipt temperature of drinking water sampling kits from the 2011 data set received at AIPH.
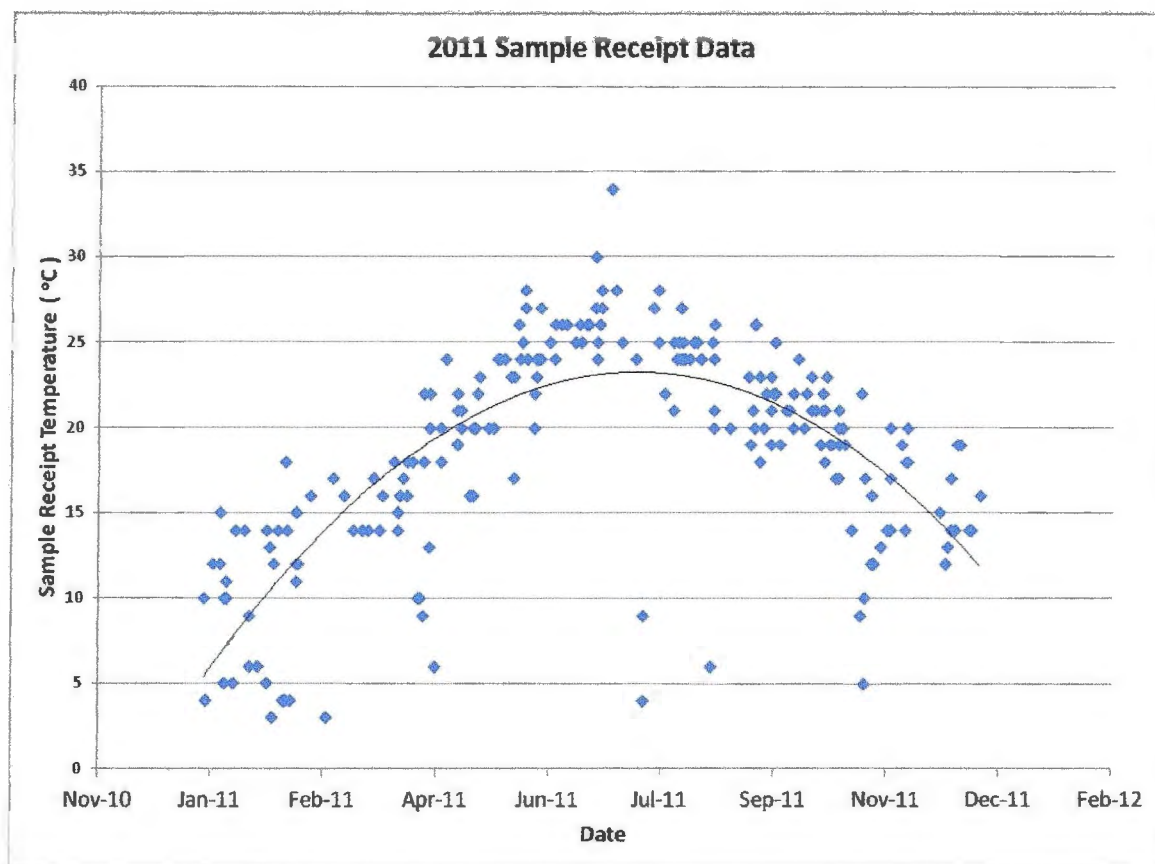
Figure 1: Receipt temperature of treated drinking water samples from OEF, 21 Jan – 28 Dec 2011.

Additionally, sample transportation from the point of collection in the Middle East or Central Asia to the US involves great distances. Pre-frozen gel packs are often not sufficient to prevent samples from exceeding the recommended storage temperature as

shipping delays may range from 1 day to several weeks (12). The USAPHC states that 21 pounds of pre-frozen gel packs can keep samples in an extra-large insulated container at 3-4°C for 120 hours (5 days) provided the container is cooled to 4° C prior to packing and shipping (34). The use of 21 pounds of pre-frozen gel packs in a sample shipping container is highly unlikely because using that amount would only allow one kit per cooler instead of the typical 3 - 4 kits per cooler. Typically 5 – 6 pounds of pre-frozen gel packs are received per cooler. Thus, if the samples remain in theater or in-transit for periods exceeding a few days, the samples will begin to assume the ambient temperature of their particular location.

As mentioned above, USAPHC has modified some of the EPA methods of analysis for specific analytes based on unique military requirements and challenges. The laboratory technician receiving the samples at AIPH provides the sample receipt conditions to USAPHC Deployment Environmental Surveillance Program (DESP) personnel who then determine if the sample will be accepted or rejected. The ideal situation would be to have all samples received at the lab within the recommended EPA MHT and storage temperature. Figure 2 has a depiction of a timeline for collection and receipt of OEH samples based on averages from the 2011 data set.
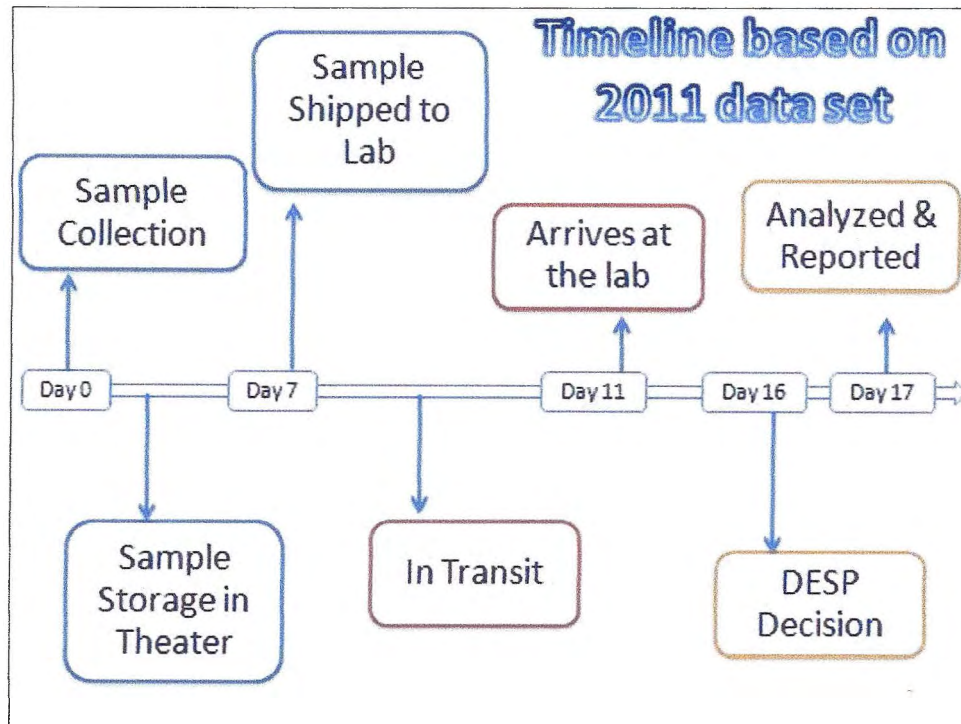
Figure 2: Depiction of the sample analysis timeline based on 2011 study data.

When samples are rejected, the decision is based on the receipt conditions for each individual sample vial outlined in the method of analysis and not the entire kit (see Figure 3), although the entire kit can be rejected.
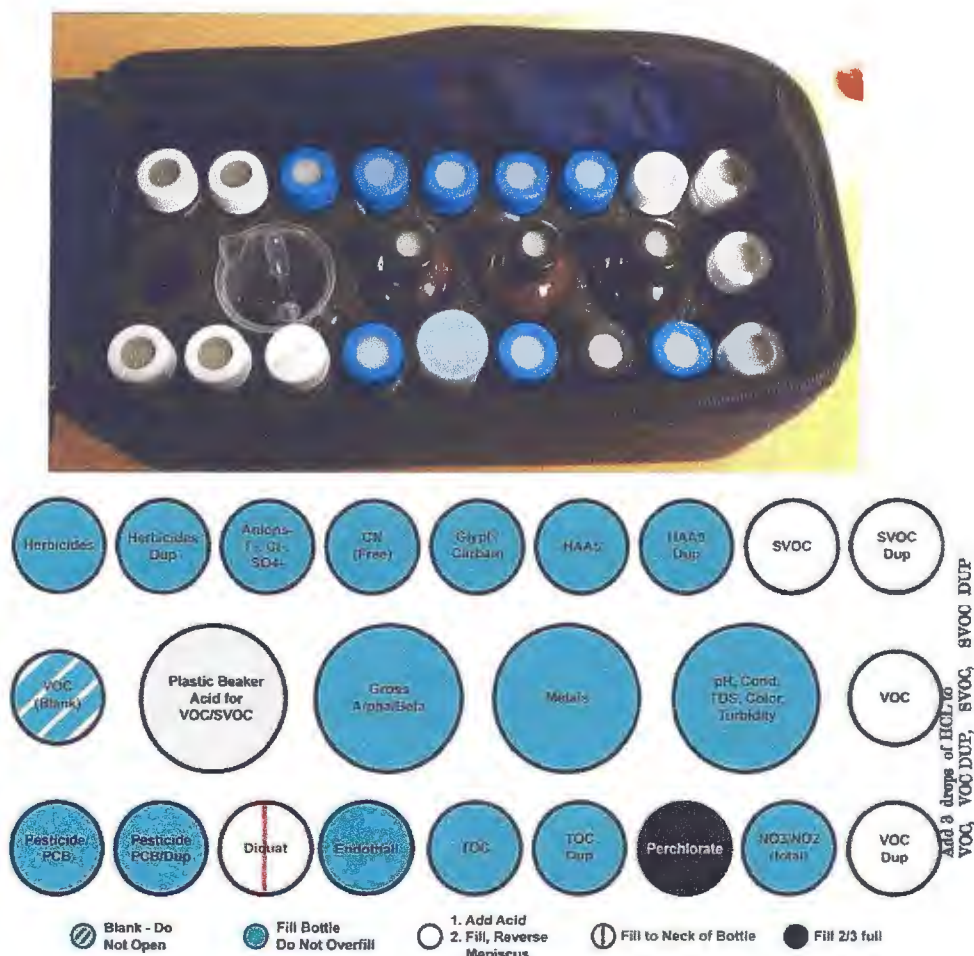
Figure 3: Currently used treated drinking water kit.

MILITARY HEALTH RELEVANCE

US Service Members continue to be deployed to various locations around the world and their health will continue to be at risk from OEH exposures. OEH exposures could be from the climate, noise, chemical and biological warfare agents, infectious diseases, physical hazards, and contaminated air, water, and soil. These exposures could be either man-made (accidental or intentional) or naturally occurring/regionally-specific (33). Drinking water is just one source of OEH exposure. Due to the receipt and analysis of some drinking water samples outside of EPA-recommended MHT and storage

temperature guidelines, the current recovery concentrations may not be an accurate reflection of the concentration at the time of collection.

OEH samples are collected, stored/packaged, and then sent to a USAPHC analytical laboratory for analysis. Once the sample analysis is complete, the results are input into the Defense Occupational and Environmental Health Readiness System (DOEHRS) where DESP personnel compare them to the Military Exposure Guidelines (MEGs). The MEGs are exposure frequency and severity-based air, water, and soil exposure limits used to evaluate the significance of a potential OEH exposure and to assess the level of health risk to deployed Service Members. However, given the potential for time delays and temperature fluctuations, if sampled compounds are temperature and/or time sensitive, the subsequent analysis may result in a lower mass yield due to chemical degradation or volatilized losses from the water. The subsequent health risk assessment and characterization of the hazard level may be inaccurate, which could underestimate the health risk to the deployed Service Member.

Findings from this study may lead to the development of new techniques and procedures to increase the confidence in deployment data, such as identifying a need to improve cold chain management techniques and shipping and handling guidelines. There could be changes made to the current process of DESP determining the acceptance or rejection of samples for analysis. Recommendations could also lead to a change in military doctrine by deploying military analytical laboratory capabilities closer to the point of collection and the development of better onsite water analysis capabilities.

HISTORICAL INFORMATION

After Operation Desert Storm in 1991, many Service Members and veterans experienced various health issues that may have resulted from potential OEH exposures (15). Force Health Protection and OEH surveillance were re-evaluated by the DoD after Operation Desert Storm/Shield (16). There were 17,142 OEH samples collected and analyzed during military operations between 1991 and 2005 (20). During this time, only 834 of these samples collected and analyzed were treated water samples. From 2006-2011, there 1,578 treated water kits received from Operations Iraqi and Enduring Freedom. The 2011 data set represents 224 documented sampling kits received over the course of an 11 month period. The 2011 period was a time consisting of the highest troop levels in Afghanistan and was just prior to the significant reduction in forces. Each of the 224 kits contained 23 treated water sampling vials to be analyzed as part of the Occupational and Environmental Health (OEH) Surveillance Program. Of the sampling kits received, only 16 of the 224 sampling kits (7%) were received at the recommended storage temperature of 4 °C +/- 2 °C (Figure 4). The 16 kits received within the recommended temperature guidelines appear to be due to the samples being collected and shipped during the winter months. The temperature of the other 208 water kits ranged from 9 °C to 34 °C. Figure 4 shows the number of kits for each temperature.
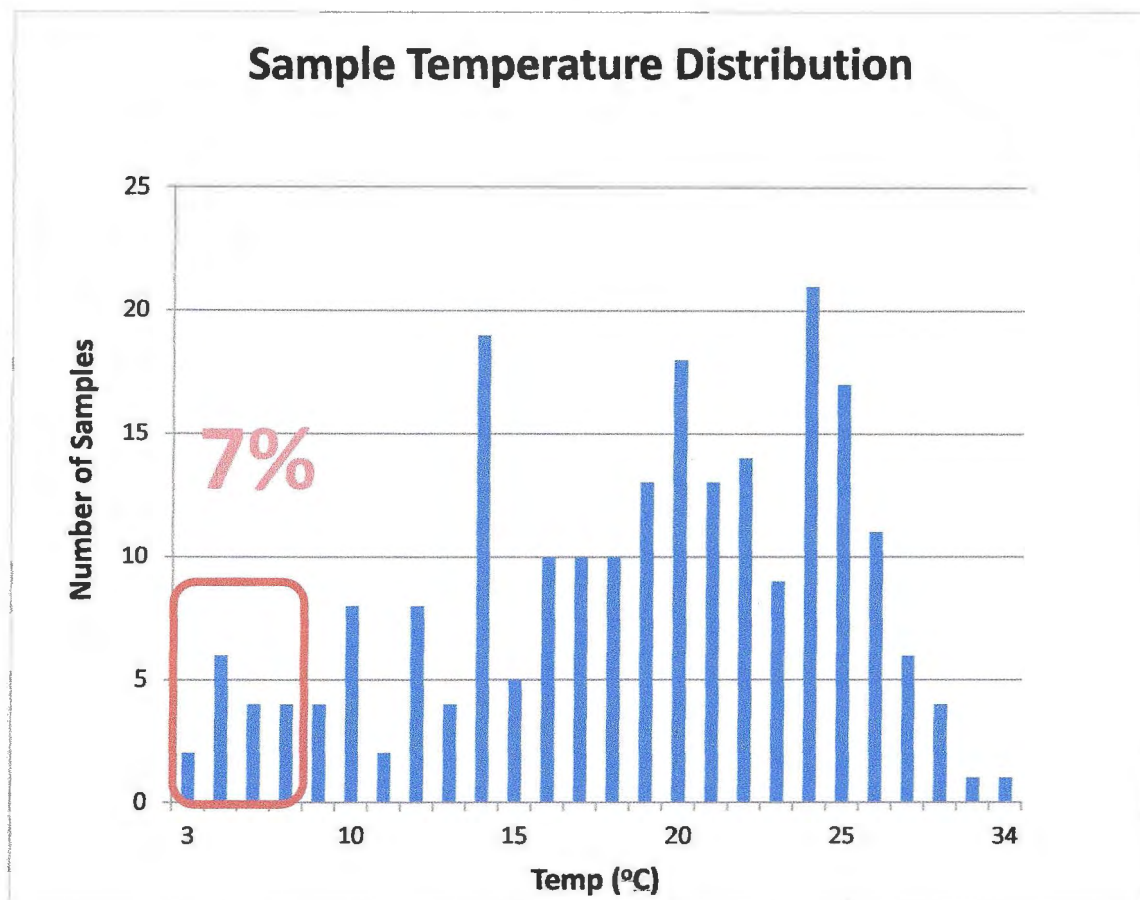
Figure 4: Water sampling kit temperature distribution of the 2011 data set received by AIPH.

On average, sample receipt occurred 11 days after collection at an average temperature of 19°C; samples were accepted by DESP an average 5 days after receipt. The majority of the samples (79%) were received within 14 days of collection (Figure 5). For a less mature theater of operations, it could take even longer to ship samples for analysis. The current method of having DESP personnel determine the acceptance or rejection of samples prior to analysis reduced the number of samples received within 14 days to 55% (Figure 6).
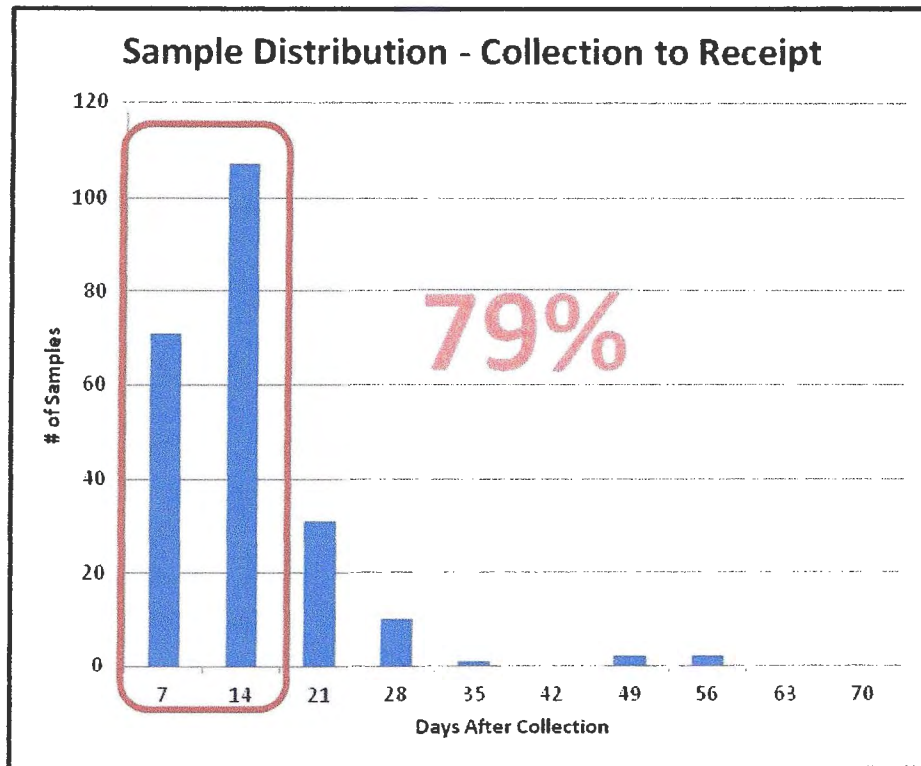
Figure 5: Water sample distribution of the 2011 data set prior to DESP review.
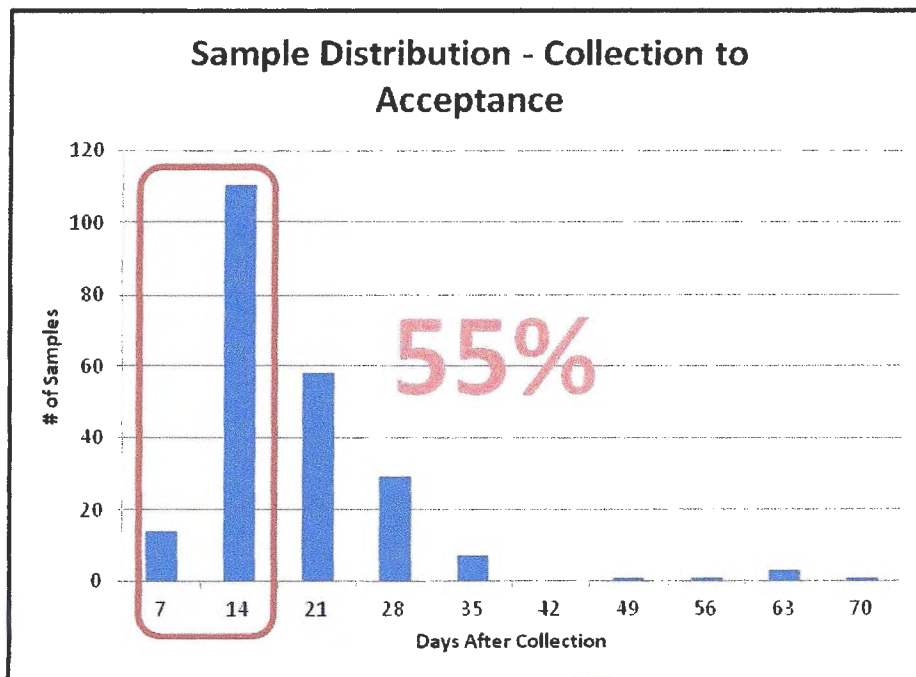
Figure 6: Water sample distribution of the 2011 data set after DESP review.

Research Objectives

This study will investigate the effects of exceeding the EPA maximum hold time, storage temperature, and the combined effect of exceeding both on samples from treated water sampling kits. The specific aims of the study are as follows:

1. Analyze contaminant-spiked water samples from a tap water matrix to measure the effect of exceeding the EPA-recommended MHT for the analytes of interest.

2. Analyze contaminant-spiked water samples from a tap water matrix to measure the effect of exceeding the EPA-recommended temperature of 4°C for the analytes of interest.

3. Investigate the combined effects of exceeding both the EPA recommended MHT and recommended temperature of 4°C for the analytes of interest.

4. Conduct a preservation study for Cyanide using chlorinated tap water and de-ionized water comparing samples with no preservative, ascorbic acid only, and ascorbic acid and sodium hydroxide to determine the best preservative to be used for CN sample collection for analysis.

The constituents evaluated in this study include Endothall, Diquat/Paraquat, Volatile Organic Compounds (VOC), Semi Volatile Organic Compounds (SVOC), Cyanide, and three metals (Antimony, Arsenic, and Mercury). These analytes were selected based on their human health significance and on their relatively short EPA-recommended hold times, except for metals. Due the logistical challenge to have samples transported and analyzed from overseas contingency operations, it is very difficult to meet the EPA guidelines. All compounds selected for this study, with the

exception of metals and cyanide, have MHTs below the average receipt time for the 224

water sampling kits from the 2011 data set, which represented the most recent data

available at the beginning of the study.

Chapter 2 – Literature Review

INTRODUCTION

The USAPHC, formerly the US Army Center for Health Promotion and Preventive Medicine (USACHPPM), is the Department of Defense's (DoD) Executive Agent for medical surveillance databases and data analysis for deployments. In July of 1996, USACHPPM established Deployment Environmental Surveillance Program (DESP) to fill the role for USACHPPM as the lead organization for Occupational and Environmental Health surveillance. The work conducted by DESP was important in addressing DoD concerns for the potential of US forces to be exposed to environmental hazards such as intentional and/or accidental releases of chemical warfare agents and toxic industrial chemicals (TIC) (16). As a result of the establishment of this program, the Environmental Surveillance Method Development project was created to develop deployable environmental sampling methodologies to support contingency operations. This project used commercial-off-the-shelf (COTS) technologies to modify the previous potable water sampling kits which were designed for garrison use by reducing the volume to make it suitable for contingency operations (16). The currently used kit (Figure 3) consists of reduced volume field expedient sampling vials, specifically designed to accommodate military logistical requirements by minimizing volume and weight (31). Reducing the volume has been determined not to hinder the analysis capability, due to the sensitivity of the analytical methods used. In addition to TICs, the kits are designed to detect chemical treatment byproducts and radioactive material.

The US Environmental Protection Agency (EPA) provides guidance within its methods of analysis on the maximum time a sample can be held prior to

extraction/analysis and the maximum storage temperature for that sample. The EPA guidelines for MHT and storage temperature are not consistent with past or present research on stability studies for VOCs (23; 27). Brian Schumacher, Ph. D., EPA Supervisory Physical Scientist commented, "While holding times may appear adequate to protect sample integrity and provide sufficient time for laboratory analysis, relevant data is sparse on individually defined holdings times and, thus, some of the holding times appear to be arbitrary and/or politically driven" (27). One example would be the EPA MHT of 14 days for VOCs stored at 4°C; past studies have shown select VOCs are stable up to 56 days when samples are preserved and stored at 4°C and 25°C (22; 23). OEH samples from overseas contingency operations are routinely out of compliance with the EPA guidelines for hold time and temperature. There are no previous stability studies of select analytes held and stored in conditions specific to those of samples received from overseas contingency operations.

The remainder of this literature review will present previous research on stability studies for selected analytes with short MHTs (Table 1). Stability studies for metals are not discussed here due to their persistence in aqueous solutions and long MHT beyond the timeframe of this study. The stability of the selected analytes is important when comparing the analytical results to the Military Exposure Guidelines (MEGs) to determine the level of health risk to US forces. The current MEGs are based on existing peer-reviewed exposure guidelines and toxicological estimates (i.e., existing health criteria) published by reputable public health and scientific organizations such as the EPA, National Academy of Science's National Research Council (NAS-NRC), and the Agency for Toxic Substances and Disease Registry (ATSDR) (33). Most of the MEGs

are set at a negligible severity level and analytical results from environmental samples may be below a specified MEG. When evaluated as part of the overall risk assessment, the risk assessment may suggest the Service Members are not at risk of exposure. However, due to degradation of the analyte or other losses which may occur if time- and temperature-based guidelines are not adhered to, the true concentration level of the respective analyte may have actually led to an exposure above a specified MEG and a potential for increased health risk.

STABILITY STUDIES – ENDOTHALL

Endothall is an active contact herbicide that prohibits protein synthesis once it has been taken up by plants (32). Endothall has low solubility in water; its primary use is to control submersed weeds. The very low octanol-water partition coefficient of Endothall suggests it is not expected to bioaccumulate or adsorb to suspended solids or sediment. Research has shown a wide range of degradation rates for Endothall, suggesting a number of environmental conditions can significantly impact its degradation rate. For example, the degradation rates of Endothall vary with treatment concentration levels and type of water matrix. In field studies conducted by R.C. Hiltibran (17), it was determined that Endothall degraded to non-detectable levels in 1 – 4 days at initial concentrations of 0.3 ppm – 10 ppm at 25°C. In a similar study by Sikka and Rice (28), Endothall degraded from pond water at 21°C – 24°C to non-detectable levels over a 36 day period at an initial concentration of 2.0 ppm. In aquaria, at 24°C ±1°C, at concentrations of 1.0 ppm and 5.0 ppm, Endothall was found to dissipate at a slower rate in clean water matrices (tap water) than in natural water matrices (lake water) containing mud, plant material, and fish (17). The sensitivity of Endothall to temperature has primarily been studied in soils; however,

little has been done on the temperature effects of Endothall degradation in water. One research effort of note demonstrated that Endothall concentrations are higher in aquatic waters during August within thermoclines and the Endothall concentrations are closer to the initial concentration when samples are taken above the thermocline. Concentrations below the thermocline were consistently lower than initial concentration (37).

Endothall is highly susceptible to microbial action. The primary fate process for Endothall in aquatic systems is biodegradation and biotransformation (30). Conditions that affect microbial action have been shown to greatly influence the degradation of Endothall in aquatic systems. Endothall persisted for 30 days when microbial degradation of Endothall was extremely slow due to weedkill in an Endothall treated lake impoundment that had considerably low dissolved oxygen concentrations (29). Once the oxygenation of the lake was restored, rapid degradation continued.

Endothall has been shown to persist in aquatic environments when conditions are optimal, despite its rapid degradation by microorganisms. Conditions affecting the degradation rate of Endothall in aquatic environments appear to be dissolved oxygen content, sediment type, and turbidity (30). Military treated drinking water samples are cleaner than the natural bodies of water where these studies were conducted. This study will use a less complex water matrix to determine the stability of Endothall.

STABILITY STUDIES – DIQUAT / PARAQUAT

Diquat is a non-selective dipyridyl contact herbicide. Dipyridyl or bipyridine is a crystalline nitrogenous base obtained by the reduction of pyridine. It is part of a family of bipyridines of which 4,4'-bipyridine is a member; 4,4'-bipyridine is the precursor for Paraquat. It is used by various industries and government agencies for control of floating

and submersed weeds. Research has shown Diquat is susceptible to microbial degradation, photochemical degradation and sorption by sediment (30). Research into the stability or persistence of Diquat has centered on its fate in natural aquatic systems where its degradation is rapid (10; 14; 18; 30; 36). Diquat was non-detect 22 days after treatment with an initial concentration of 1.5 ppm in a simulated lake impoundment at 25°C. Diquat persisted for four days after treatment with initial concentrations of 0.62 ppm (10), and displayed rapid degradation approaching zero concentration 30-33 hours after treatment with initial concentration of 1 ppm in pond water at 19°C (21). After 12 days of treatment with an initial concentration of 2.5 ppm in pond water at temperatures ranging from 25.5°C – 30°C and 6.7°C – 12.2°C, only trace amounts of Diquat were detectable (14; 36). In a comparison of seasonal persistence, Hiltibran *et al.* (18) found that when the water was clearer in the Spring, persistence was approximately seven days greater than in the Fall when the water was more turbid. Diquat readily binds to plant materials; its effectiveness is greatly reduced by muddy or turbid water (9; 32). The degradation rate of Diquat in aquatic systems is heavily influenced by the amount and availability of sorbent material like sediment, aquatic plants, and suspended particulate matter in the aquatic system (10; 14; 18; 30; 36). The persistence of Diquat varied from study to study and this can be attributed to different characteristics of the aquatic system and treatment concentrations along with the varying environmental conditions.

As with Diquat, Paraquat is also a non-selective dipyridyl contact herbicide. The persistence of Paraquat in water is largely determined by the concentration of the herbicide and the complexity of the water matrix. In ponds with comparable water quality except for turbidity, the persistence of Paraquat presented with different results.

Paraquat persisted for 23 days in pond water at 28 °C that had lower turbidity of less than 25 Jackson Candle units compared to six days in pond water at 8°C where the turbidity averaged 42 Jackson Candle units (14). Paraquat persistence was not affected by water temperature. In growth pools containing 1.0 ppm, 2.0 ppm, and 3.0 ppm Paraquat concentrations, the concentration levels of Paraquat after 12 days were between 0.8 ppm and 0.3 ppm with water temperatures at 25°C – 27.8°C (36). Paraquat is slightly more persistent than Diquat under similar environmental conditions (10). At an initial concentration of 2.5 ppm for Diquat and 2.1 ppm for Paraquat applied to the same pond, Diquat was non-detect after 30 days while Paraquat was non-detect after 38 days (14). As with Endothall, military drinking water samples are clearer and should be free of sediment, microbes, plants, and other materials that would degrade Diquat and Paraquat. The stability in this study may be affected by other constituents not currently known to degrade either of the contact herbicides.

STABILITY STUDIES – VOLATILE ORGANIC COMPOUNDS

Volatile organic compounds (VOCs) are organic compounds with high vapor pressure at 25°C, defined as possessing vapor pressures greater than 10^{-4} mm Hg at standard temperature and pressure. The research on the long-term stability of VOCs in water is limited. There is uncertainty surrounding the quality of results from VOCs held beyond the 14 day MHT for trace concentrations because there is little information on the stability of VOCs at low concentrations. Research efforts conducted by Maskarinec *et al.*, (23) and Love, *et al.*, (22) investigated the validity of the recommended EPA MHT for VOCs of 14 days to determine if the pre-analytical hold times could be extended.

Maskarinec et al., (23) conducted foundational research on the stability of VOCs in environmental water samples. Their study used initial concentrations of 50 µg/L and 500 µg/L while investigating preserved and unpreserved samples of 17 different VOCs in distilled water, groundwater, and surface water. In the study, the ASTM D4841 method to determine MHT was compared to the method employed by Environmental Science Engineering, Inc. (ESE). At the higher concentration studied, the ASTM method resulted in increased MHTs in greater than 70% of unpreserved samples, while both methods produced similar increases in MHTs at the lower concentration (23). The authors noted the higher concentration (500 µg/L) yielded more instances of longer MHT than the lower concentration (50 µg/L), but found them not to be statistically significant. At 25°C, 88-94% of the unpreserved samples showed stability of at least 19 days for the ASTM method and 76-88% of at least 16 days using the ESE method. However, the results appeared to be matrix and concentration dependent. Based on the study results, when the samples are preserved with HCl and held at 4°C, the data show preserved VOC samples of the 17 VOCs studied could be held at least 56 days prior to analysis (23).

Love, et al.,(22) conducted research on the long-term stability of 87 VOCs in environmental water samples using a much lower trace concentration (0.5 µg/L) than Maskarinec et al., in an attempt to be more representative of what is found in the majority of the environmental samples received by the US Geological Survey National Water-Quality Laboratory. The study used surface water and groundwater stored at 4°C, preserved with HCl, and a target initial concentration of 0.5 µg/L. At the initial target concentration, approximately 90% of the VOCs studied showed stability beyond the 14

day EPA MHT and approximately 80% displayed stability that ranged from 37-56 days; while 93% appeared to be unaffected by the matrix (22).

There is no widely accepted standard on how to collect water samples for VOC analysis in regards to the method to fill the collection vial. Different environmental companies, organizations, and professionals have put forth different methods supplying their own justification without citing any specific study to validate their reasoning (25). The rate at which the sample collection vial is filled with the water sample and the pour technique (top-pour, side-pour, and bottom-fill) during sample collection has been shown to affect the recovery concentration of VOCs. The top-pour technique placed the discharge tube about 1 inch above the vial opening and allowed the water sample to vertically fall into the vial (25). The side-pour technique allowed the water sample to smoothly flow down the inside wall of the vial by placing the tubing just inside the vial opening and tilting the vial at a 30 – 45 degree angle (25). The bottom-fill technique placed the tubing near the inside bottom of the vial and the vial was filled with the tube slightly below the surface of the water. The tube was slowly removed as the vial filled by lowering the vial (25). It has been demonstrated in controlled laboratory experiments that the best method is to fill the vial from the bottom with a tube submerged in the sample; it yielded a 6 – 14% greater recovery compared to the side pour and top pour methods. In regards to fill rate, a fast fill rate yielded a higher recovery (6 – 15%) than the slower fill rate (25).

STABILITY STUDY – SEMI-VOLATILE ORGANIC COMPOUNDS

Semi-volatile organic compounds (SVOCs) are organic compounds with relatively moderate vapor pressure, defined as possessing vapor pressures between 10^{-4}

mm Hg and 10^{-11} mm Hg at standard temperature and pressure. Two of the classes of SVOCs tested by the Army Institute of Public Health (AIPH) laboratory as part of the drinking water sampling kits are Polycyclic Aromatic Hydrocarbons (PAH) and Phthalates. Research conducted by Gallott *et al.*, (11) used the method from ASTM 4841 to calculate the MHT. The study was conducted over a 41 day period investigating the stability of 12 PAHs and found nine of the 12 PAHs were stable and remained within tolerable range variations (*TR*) for the duration of the study period when held preserved in a saline water matrix. Only three PAHs were outside tolerable range variations; of these three, two were stable up to 22 days, and one for 30 days.

There were no stability studies for phthalates found in the literature. The majority of the research found on phthalates was related to chemical leaching from plastics and the risks phthalates pose to humans. Phthalates are endocrine disrupting chemicals; they are used as solvents and in plastics manufacturing. Plastics containing phthalates are often used in applications involving building materials, clothing, cosmetics, perfumes, food packaging, toys, and vinyl products; they are also found in medical devices, blood transfusion bags, and IV bags (7). Some of the products used by the US military contain phthalates. During Operation Enduring Freedom there was concern that bottled water stored in direct sunlight would release harmful chemicals such as phthalates from the plastics into the water; Greifenstein *et al.* (13) conducted a 28 day study to determine the amount of SVOC phthalates that liberate or migrate from Polyethylene Terephthalate (PET) containers and found four phthalates present on Day 0, Diethylphthalate, Butylbenzylphthalate, Di(2-ethylhexyl)phthalate, Di-n-butylphthalate. They found low (near Method Detection Limit - MDL) concentrations of Diethylphthalate on Day 7 at

23.5°C and 60°C and Butylbenzylphthalate nearly twice the MDL on Day 7 at 48.8°C were detected during the study. Also, concentrations of Di(2-ethylhexyl)phthalate were 0.14 μg/L - 1.30 μg/L and concentrations of Di-n-butylphthalate were 0.16 μg/L - 0.30 μg/L. They concluded the phthalate migration was from the plastic tubing, pipes, and gaskets, not the bottles (13). Although Greifenstein *et al.* did not conduct a stability study; their research demonstrates that SVOC phthalates can be detected at elevated temperatures.

STABILITY STUDIES - CYANIDE

Free cyanide exists as cyanide anion and hydrogen cyanide in a gaseous or aqueous state. Hydrogen cyanide (HCN) is soluble in water and the solubility decreases as the temperature of the media increases (19). Paulson (26) conducted stability studies of cyanide at 2°C and 21°C over a one hour period collecting data at 10 minute intervals. The study was conducted in twice distilled water; no preservative was mentioned in the research. The results showed a 48.8% degradation of cyanide at 2°C compared to 63.3% at 21°C. Cyanide (CN) ions in water hydrolyze to produce hydrogen cyanide which has a vapor pressure of 732 mm Hg at 25°C compared to 5 mm Hg at 25°C for bromoform (VOC), 1.0×10^{-7} mm Hg at 25°C for di(2-Ethylhexyl phthalate) (SVOC), and 0 mm Hg at 25°C for antimony (metal). Comparing this to the average received temperature of 19°C for water samples from overseas contingency operations, HCN is expected to volatilize from the samples proportional to temperature as it would from any sample at 19°C. Based on this, the CN recoveries from overseas contingency operations may not accurately represent the true concentration when samples are received above the method storage temperature and MHT guides.

LITERATURE REVIEW SUMMARY

Based on this literature review, it is suggested that the studied VOCs and SVOCs (PAH) MHT could be extended using the definition of stability as applied by ASTM D4841. VOCs have also been shown to be stable beyond the MHT when preserved with HCl at 4°C and at 25°C. The water matrix appears to be the major difference between the studies from the literature review and the research conducted for this study. The water matrices for the VOCs and SVOCs could be as complex as or more complex than the tap water used for this study. Using a less complex water matrix would suggest equal or greater stability compared to the literature review. Previous studies have shown the persistence of Endothall, Diquat, and Paraquat samples is matrix dependent; these herbicides were not shown to be sensitive (in the various matrices studied) to the temperatures and durations studied. The studies used in this literature review used natural bodies of waters which are for more complex water matrices than the use of tap water in this research. In regards to CN stability, any sampling for CN analysis would need to account for the many interfering compounds to obtain the most accurate results.

Chapter 3 – Materials and Methods

MATERIALS

For all time and temperature based experiments in this study, water samples were prepared using tap water from the AIPH laboratory at Aberdeen Proving Ground, MD. The cyanide study was modified to use de-ionized (DI) water and untreated private well water because of non-detection of the CN ion possibly caused by interfering constituents in the tap water. A tap water matrix was used because it is considered treated and the drinking water samples received from overseas contingency operations are also treated, with the full understanding that the tap water from the AIPH laboratory may have substantially different properties than treated groundwater from a foreign country. These differences may impact the degradation of analytes in this study. The only characterization done on the tap water was for free available chlorine, which was measured at 0.8 ppm. Commercially pre-cleaned and preserved 40 ml amber glass sampling vials (Scientific Specialties Services, Inc. and Scientific Products, LLC – Diquat/Paraquat only) with an open top septum cap identical to the vials from the treated water deployment sampling kit were used to collect and prepare the water samples for analysis, except for metals. Commercially pre-cleaned and preserved 125 ml amber glass bottles (Scientific Specialties Services, Inc.) with open top septum cap were used for metals sample collection. Each vial/bottle contained commercially pre-loaded preservatives; the vials were purchased with the desired amount of preservative in each vial (Table 2).

Analyte	Target Conc (µg/L)	Preservatives	Sample Analysis Points (Days)
VOC	40	25 mg Ascorbic Acid; pH<2 w/ 1:1 HCl (3 drops)	0, 8, 14, 28, 42
SVOC	1.25	3 mg Sodium Sulfite; pH<2 1:1 HCl (3 drops)	0, 8, 14, 28, 42
Endothall	500	4 mg Sodium Thiosulfate	0, 8, 14, 28
Diquat / Paraquat	50	4 mg Sodium Thiosulfate; pH<2 w/1:1 H2SO4 (2 drops)	0, 3, 8, 14, 28
Cyanide	50	pH>12 w/ 10N NaOH (4 drops)	0, 7, 14, 21, 30
Mercury	5	pH<2 w/ 1:1 HNO3 (8 drops)	0, 28, 42
Antimony and Arsenic	50	pH<2 w/ 1:1 HNO3 (8 drops)	0, 28, 42

Table 2: Target concentration, preservative, and sample analysis points for all analytes used in this study.

The study water samples were prepared by spiking a known amount of analyte into a predetermined water volume to reach the target analyte concentration. The vials/bottles were filled using a 500 ml beaker. The side pour technique was used at about a 250 ml/min flow rate. After filling, all samples except for VOCs and SVOCs were capped for storage. The pH for the VOC and SVOC samples was adjusted below 2 with the appropriate amount of acid then capped for storage (Table 2).

Three temperature conditions (4°C, 25°C, and 39°C) were used to model possible receipt temperatures for samples from overseas contingency operations. The 4°C temperature was selected because it is the storage temperature stated in each method of analysis except for metals which have no temperature storage requirement. The 25°C and 39°C temperature was selected to represent temperatures that samples could be exposed to during shipment in the summer months. Although the actual temperature profile data

during shipment was not available, summer temperatures in Afghanistan during Operation Enduring Freedom (OEF) could be as high as 41°C depending on location and summer temperatures in Iraq during Operation Iraqi Freedom (OIF) could be as high as 44°C (24). The samples were stored in either a refrigerator or incubator at the Uniformed Services University of the Health Sciences (USUHS) Division of Occupational and Environmental Health Sciences (OEHS) water lab. The refrigerator (General Electric) temperature was at 4°C. The temperature varied from 2-5 °C, as noted from observed temperature readings taken two to three times per week. The incubator temperatures were set at 25°C (Fisher Scientific) and 39°C (Precision - THELCO Laboratory Oven) and with a variation of +/- 2°C, theses temperatures were also observed two to three times per week. Temperatures were held constant over time while real world samples may initially be stored at 4°C, but would likely experience temperature changes from ambient temperatures once the pre-frozen gel packs begin to thaw.

Storage times (Analysis points – See Table 2) were developed to bracket the recommended EPA MHT (except for Arsenic and Antimony), encompass the average receipt date, and extend at least 14 days beyond the MHT to study degradation over specified time intervals. The analysis points in Table 2 had to be shifted for a few samples due to conflicts with federal holidays. The study was not extended past 42 days because a review of the 2011 data set showed only two drinking water kits were received beyond 42 days so it was proposed these kits may have been lost, mislabeled, or delayed in customs. The EPA MHT, storage temperature, and method of analysis for each analyte are presented in Table 1.

Sample analyses were conducted at the AIPH Laboratory at Aberdeen Proving Ground, MD or sent to a contract laboratory. As stated previously, AIPH uses modified EPA methods of analysis to meet the unique military logistical requirements involved with OEH sample collection. There were major modifications to the SVOC and Diquat/Paraquat methods, minor modifications to cyanide and Endothall, and no modifications were made for VOCs and metals. In all cases the laboratories were ISO 17025 accredited through the National Environmental Laboratory Accreditation Conference and American Association for Laboratory Accreditation.

METHODS

In applying the method outlined in ASTM D4841, equation (1) was used to determine the appropriate number of analysis replicates (n) to be used for each analyte at the various analysis points. The example below is the n determination for Toluene. The method recommends 10 initial replicates be used to determine the number of analysis replicates. For this study, three initial replicates were used. The use of only three initial replicates affected the standard deviation used to calculate the relative standard deviation. The standard deviation is more sensitive to large differences in values when a small sample set is used. A large standard deviation leads to a large RSD and n value determination. The purpose of the research was not to determine a new MHT for each analyte rather it was to determine the chemical stability of each analyte as they exceeded the EPA MHT and recommended storage temperature. Therefore, three replicates were used because three samples were sufficient to determine if the stability was affected by time or temperature when conducting the research.

$$n = \left(\frac{t\,RSD}{D}\right)^2 \qquad (1)$$

where:

n	$=$	number of analysis replicates required,
t	$=$	Student's T test based on number of samples,
RSD	$=$	Relative Standard Deviation, %, and
D	$=$	Maximum variation from mean concentration to be tolerated, 15%.

Example:

Toluene Day 0 recovery concentrations (µg/L): 40, 39, 38

n	$=$	3
t	$=$	9.925
RSD	$=$	2.56 (determined from Day 0 recovery concentrations)
D	$=$	15

$$n = \left(\frac{9.925\,(2.56)}{15}\right)^2 = 3$$

If the calculated n value is a decimal, then round up to the next whole number.

Applying statistical determinations from Gerald Van Belle's *Statistical Rules of Thumb* to determine sample size, a sample size of three per condition is sufficient to detect degradation of 25% if the coefficient of variation (CV) is 10%, assuming 80% power and 5% 2-sided significance level (35). Three replicate spiked water samples were prepared for each analyte and analyzed at each analysis point outlined in Table 2. There were a few n value calculations that were higher than the three replicates used in this study. As stated previously, three replicates were sufficient to determine if non-compliance with the time and temperature guidelines for the water samples reduced the stability of the analytes.

As part of quality control, trip blanks were also prepared and analyzed with each analysis point. Matrix spikes (MS), matrix spike duplicates (MSD), laboratory control spikes (LCS), and laboratory control spike duplicates (LCSD) were also prepared for each batch of samples submitted for analysis. Each of these quality control measures had recoveries within the acceptable recovery limits, thus, the instruments and lab technicians were performing to standard.

To measure the effect of exceeding time, temperature, and time and temperature combined, the method outlined in ASTM D4841 was applied to develop graphs for comparison and evaluation. This method allows the chemical stability to be measured in number of days it takes the mean recovery concentration to fall below the lower limit of a 99% confidence interval from a two-sided T-Test which is referred to as the tolerable range variation. The measured effect is referred to as the MHT or SMHT. In applying the ASTM D4841 method, the mean concentration for each analysis point per condition was calculated for each analyte. Using the 99% confidence interval of a two-sided T test ($t = 9.925$), the lower limit of the tolerable range (*TR*) was also calculated using the equation (2) to determine the tolerable range variation (*TRV*). The lower limit of the *TR* is calculated by subtracting the *TRV* from the Day 0 mean concentration.

$$TRV = \pm \frac{t\,s}{\sqrt{n}} \qquad (2)$$

where:

TRV	=	Tolerable range variation
t	=	Student's T test based on number of samples
s	=	Standard deviation
n	=	Number of replicate samples.

Example:

Toluene Day 0 recovery concentrations (μg/L): 40, 39, 38

TRV = -5.73 (Lower Limit)
t = 9.925
s = 1 (determined from Day 0 recovery concentrations)
n = 3

$$TRV = -\frac{(9.925)(1)}{\sqrt{3}} = -5.73$$

To calculate the lower limit of the TR, subtract the TRV from the mean of the Day 0

recovery concentration:

$Day\ 0\ mean$ = 39
TRV = -5.73
TR = 33.27

$$TR = 39 - 5.73 = 33.27$$

After calculating the TR it was normalized to the Day 0 concentration and used to create

the graphs, percentages are presented in the graphs.

$$33.27/_{39} = 0.853\ or\ 85.3\%$$

The recovery concentrations were normalized to the Day 0 recovery

concentrations producing a recovery percentage and mean recovery percentage. A graph

of the mean recovery percentage versus time was plotted for each analyte. A best fit

curve was drawn and overlaid with the tolerable range to determine the difference, if any,

between the respective analyte results from the varying analysis points examined and the

results from samples analyzed within acceptable EPA guidelines. At the point where the

best fit curve intersects the *TR*, a vertical line was drawn perpendicular to the x-axis to determine the SMHT.

The CN study was modified due to non-detection of the CN ion in the spiked water samples using the tap water matrix and NaOH only as the preservative. Untreated well water and DI water were used in place of tap water due to the non-detections. The CN samples were prepared as prescribed above. An additional preservative study was also conducted using different CN preservatives. Method OIA 1677 suggests using ascorbic acid as a preservative and NaOH to maintain pH however AIPH currently uses NaOH only. The preservative study was designed to use tap water for the water matrix with three preservative conditions (no preservative, ascorbic acid only, and ascorbic acid with NaOH); each sample was held at 4°C. The samples were prepared as prescribed above except for the samples that were preserved with ascorbic acid. These samples had the ascorbic acid added to the spiked water solution then the appropriate vials were filled. The samples with no preservative and ascorbic acid only used commercially pre-cleaned 40 ml glass sampling vials with no preservatives added.

To determine the effects of exceeding the time requirement each analyte was evaluated at analysis points beyond the EPA MHT at the 4°C temperature condition. If the analyte was determined to have a SMHT greater than the EPA MHT, then it may possibly be chemically stable beyond the EPA MHT. To determine the effects of exceeding the recommended temperature, the 4°C graph was visually compared to the 25°C and 39°C graphs. If the analyte had a SMHT greater than or equal to the EPA MHT, then it may be chemically stable at the storage temperature used during the study for that particular analyte. To determine the combined effect of exceeding the EPA MHT

and recommended temperature was the most challenging because there was no straight forward comparison to be made so each analyte was evaluated at each elevated temperature at analysis points beyond the EPA MHT. If analyte had a SMHT greater than the EPA MHT, then it may possibly be chemically stable beyond the EPA MHT and at temperatures used during the study for that particular analyte.

Chapter 4 - Results and Discussion

INTRODUCTION

ASTM D4841 was used to determine the chemical stability of analytes using the maximum holding time by plotting recovery concentrations vs. time (2). The method involves drawing a best-fit curve based on the mean recovery concentrations from each analysis point (2). A horizontal line representing the lower interval of the 99% confidence interval (Tolerable Range) is then drawn on the same graph. At the point where the best-fit curve intersects the Tolerable Range (*TR*), a straight line is drawn from the point of intersection perpendicular to the x-axis to determine the MHT. Instead of using mean recovery concentrations, mean recovery percentages were used to develop graphs to determine the chemical stability. This method was applied to the data from this study to determine the Study MHT (SMHT). The best-fit curve was usually not a smooth curved line; it was often a 2nd or 3rd degree polynomial that produced a coefficient of determination (R^2) closest to 1. The degree of polynomial was increased to get closest to one without equaling one. Using 2nd and 3rd polynomials made it difficult to directly determine the stability of the analyte due to the non-uniform nature of the best-fit line. In these instances a range for the SMHT was given based on the most recent data point above the *TR* and the 1st data point below the *TR*.

The *n* value determinations are listed in Table B1 for each analyte. Some of the analytes had higher *n* value determinations than was used in the study to determine the *TRV* which was used to calculate the *TR*. The analytes with the highest RSD values also have the highest *n* values. The highest *n* values were for Endothall, Cyanide, and

SVOCs; they ranged from 10 – 69. Arsenic, Bromoform, and Dibromochloromethane had n values that ranged from 4 – 5. The remaining analytes had n values equal to or lower than three. By using only three initial samples, the RSD was sensitive to large internal differences between the three replicate samples which increased the RSD and n values. Endothall was the highest at 69. Based on the n value determined from the initial three replicate samples, 69 samples of Endothall would need to be analyzed at each analysis point to determine a new MHT. Since the purpose of this study was determine chemical stability beyond the EPA MHT and recommended storage temperature, the 69 samples were not necessary. However, using the calculated number of samples would have increased the confidence in the results of the study for those analytes with n value determinations above three.

The remainder of this chapter will present the findings from this study. The results of the modified ASTM D4841 analysis are presented in Table 3, which provides the Study MHT to compare with EPA guidelines. Appendix A contains graphs with the best-fit curves for all analytes used in this study. Appendix B contains the mean recovery percentage for each analyte at each analysis point and temperature condition.

| Analyte | Category | Study Maximum Holding Times (SMHT) | | | EPA MHT |
		4°C	25°C	39°C	4°C
CN - Well	Ion	42	42	21 - 28	14
CN - DI	Ion	42	42	7 - 14	14
Endothall	Herbicide	28	28	28	7
Diquat	Herbicide	42	28 - 42	28 - 42	7
Paraquat	Herbicide	42	42	42	7
Mercury	Metals	42	42	42	28
Arsenic	Metals	42	42	42	180
Antimony	Metals	42	42	42	180
1,1,1-Trichloroethane	VOC - Solvent	42	<8	<8	14
Chloromethane	VOC - Solvent	42	<8	<8	14
Tetrachloroethene (PCE)	VOC - Solvent	<8	<8	<8	14
Trichloroehtene (TCE)	VOC - Solvent	42	<8	<8	14
Benzene	VOC - Solvent	28 - 42	<8	<8	14
Toluene	VOC - Solvent	42	8 - 14	<8	14
Bromoform	VOC - THM	<5	<5	<5	14
Bromodichloromethane	VOC - THM	<5	<5	<5	14
Chloroform	VOC - THM	42	Inc	Inc	14
Dibromochloromethane	VOC - THM	<5	<5	<5	14
Butylbenzylphthalate	SVOC - Phthalate	42	42	42	14
Di(2-ethylhexyl)phthalate	SVOC - Phthalate	42	42	42	14
Diethylphthalate	SVOC - Phthalate	42	42	42	14
Dimethylphthalate	SVOC - Phthalate	42	42	42	14
Di-n-butylphthalate	SVOC - Phthalate	42	42	42	14

Table 3: The Study and EPA MHTs for analytes presented in the study; green, SMHT > EPA MHT; red, SMHT < EPA MHT; white, inconclusive.

ENDOTHALL

The results from the 28 day Endothall stability study are presented in Table 3 and Graphs 1 - 2 in Appendix A. When calculating the *TR* for Endothall, the standard deviation was large which caused the *TRV* to be high (see Table B1). The high *TRV* led to a low *TR* for stability determination. At the recommended temperature of 4°C,

Endothall had consistent degradation until day 28 where the mean recovery percentage was greater than Day 14 (see Figure 7).
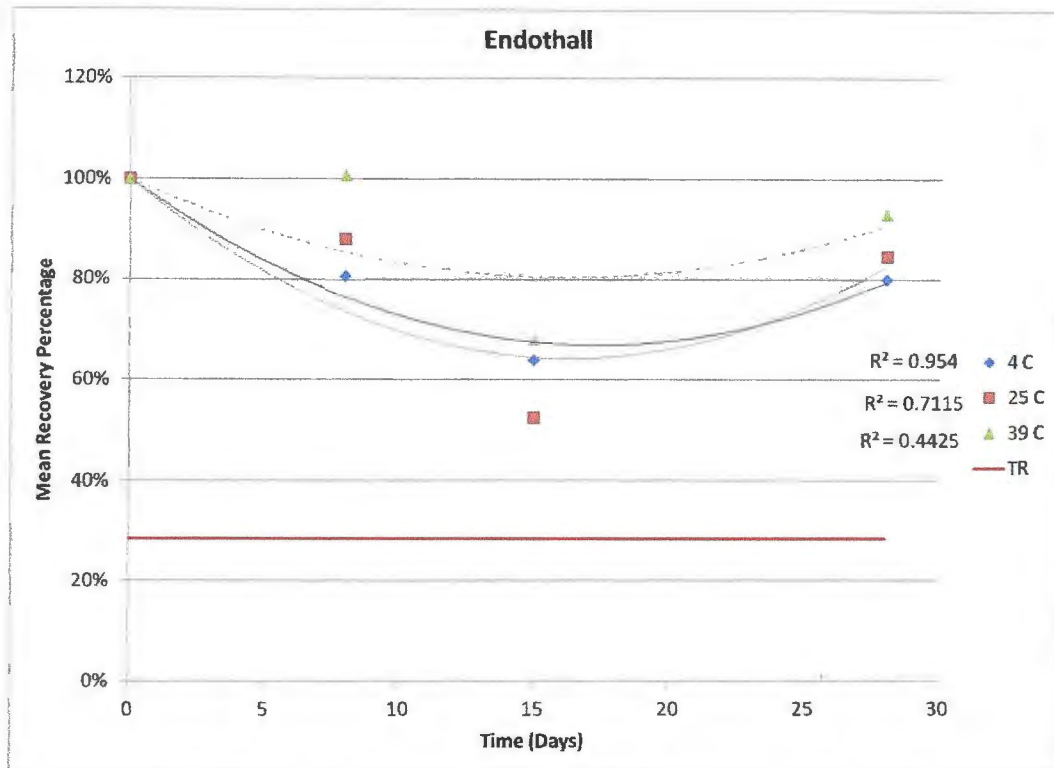

Figure 7: Graph of Endothall stability results over 28 day study period.

Exceeding the EPA MHT alone did not appear to hinder the stability of Endothall. The mean recovery percentage never dropped below the TR. In a review of the quality control (QC) results for Day 14, the two QC samples were lower than Day 0 and Day 8 QC results by 10 – 40%. Base on EPA Method 548.1, potential major interferences are dissolved calcium, sulfate, and magnesium. These ions are the only known ions to demonstrate the ability to interfere with Endothall recover when using this method. Also, based on EPA Method 548.1 the most probable interferences are organic acids and phenols retained by the Gas Chromatograph (GC) column which may explain the

variation in results if the GC column was replace due to maintenance between analysis points. The SMHT was determined to be 28 days compared to the EPA MHT of seven days. At the elevated temperature conditions, the results were similar with consistent degradation until day 28 possibly for the same reasons as stated above. The SMHT at each elevated temperature was determined to be 28 days. Reviewing the data points from Day 8 and beyond at each temperature condition, the degradation never fell below the tolerable range limit. The results show Endothall SMHT is 28 days across each temperature condition which is beyond the EPA MHT. The effects of Time, temperature, nor the combination of the two did not appear to be a factor in the degradation of Endothall. Endothall stability is greatly influenced by the constituents within the water matrix. The stability of Endothall suggests the tap water matrix was absent the known degradation components of Endothall; microbes, sediment, organic plant material, etc.

DIQUAT/PARAQUAT

The data from the 42 day Diquat / Paraquat study is presented in Table 3 and in Appendix A Graphs 3 - 4 for Diquat and Graphs 5 – 6 for Paraquat. At the EPA recommended temperature and reviewing the effect of time on the analytes, the mean recovery percentage of Diquat never falls below the TR, but equals the TR at Day 28 then rises on Day 42 (see Figure 8). The SMHT for Diquat at 4°C was determined to be 42 days.
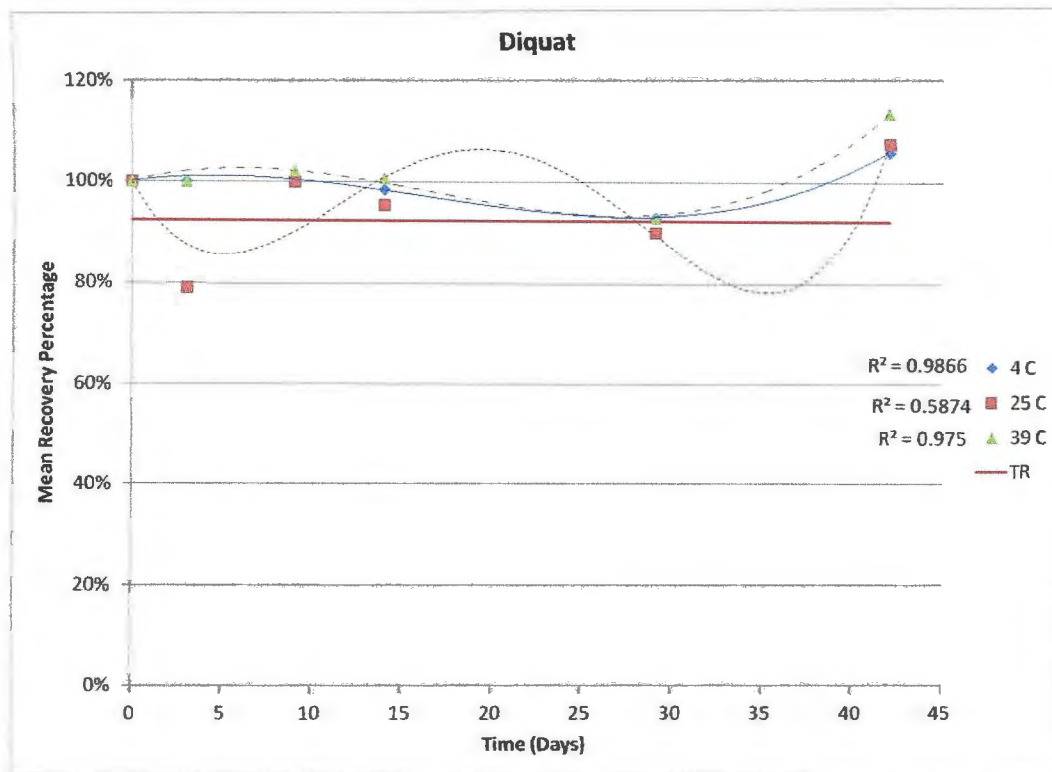
Figure 8: Graph of Diquat stability results over 42 day study period.

Paraquat has no individual or mean data points that fall below the TR, however, the mean recovery percentage increases from Day 28 to Day 42. Paraquat was determined to have a SMHT of 42 days at 4°C. At the elevated temperatures both again show increasing recovery between Days 28 and 42. However, on Days 3 and 28 the mean recovery percentage for Diquat falls below the *TR*. The Day 3 mean recovery percentage has one data point well below the *TR* and heavily influences the mean. The other data points on Day 3 are above the *TR*. On Day 28 there are two individual data points below the *TR*. The SMHT of Diquat at 25°C and 39°C was determined to be greater than 14 days but less than 28 days. Paraquat has no individual data points or mean data points that fall below the *TR* at either elevated temperature. The stability of Paraquat appears to be at least 42 days. The effect of time and temperature does not

appear to increase the degradation for either analyte. The recovery of Diquat and Paraquat was consistent across each temperature condition. The chemical stability of Diquat appeared to be at least 42 days at 4°C and greater than 14 days but less than 28 days at the elevated temperature conditions while the stability of Paraquat appeared to be at least 42 days at each temperature condition. The graphs for Diquat and Paraquat were visibly evaluated. Time, temperature, and the combination of time and temperature did not appear to reduce their chemical stability; their mean recovery percentages never fell below the *TR* before the seven day EPA guideline. As with Endothall, the stability of Diquat and Paraquat are greatly influenced by the constituents within the water matrix. The stability of Diquat and Paraquat suggests the tap water matrix was absent the known degradation components of Diquat and Paraquat; microbes, sediment, organic plant material, etc.

METALS

The metals data is presented in Table 3 and Graphs 7 - 12 in Appendix A. Reviewing the data for Mercury and Antimony, the mean recovery percentage was higher at Day 28 than at Days 0 and 42 across all three temperature conditions. There were no individual data points below the *TR* at either analysis point or temperature condition (see Figure 9).
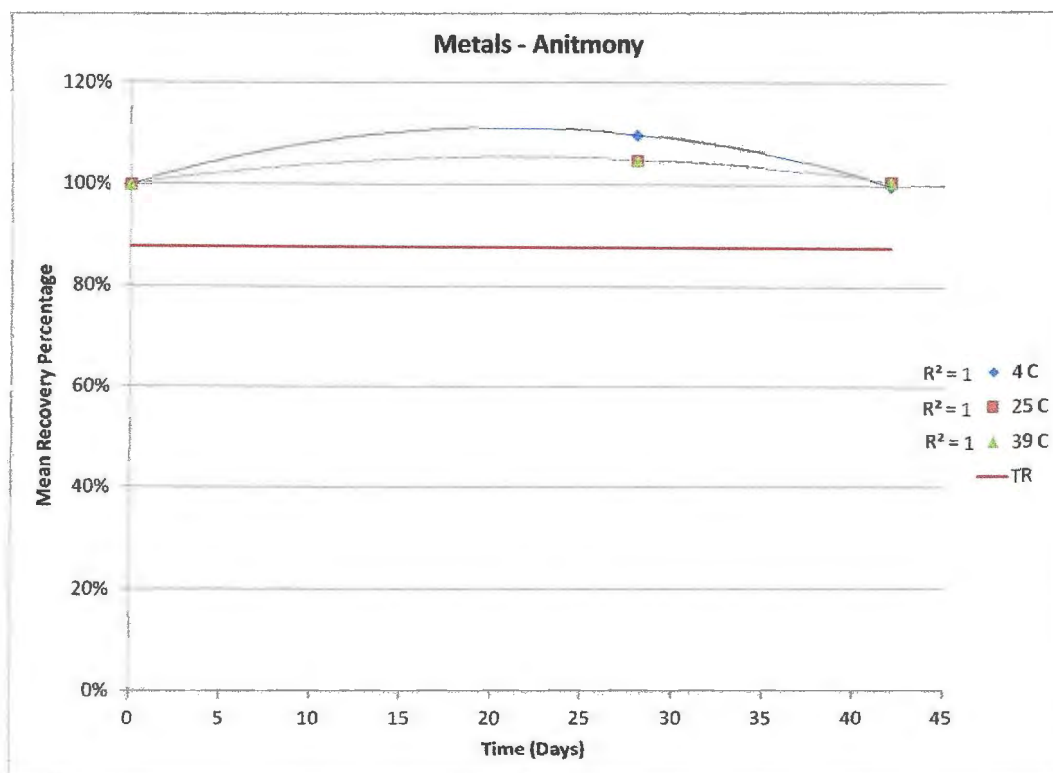
Figure 9: Graph of Antimony stability results over 42 day study period.

Across all three temperature conditions, the mean recovery percentage for Arsenic deceased from Day 0 to Day 28, but increased from Day 28 to Day 42. There were no individual data points below the *TR* at either analysis point or temperature condition. The three metals studied appeared to be consistently stable throughout the 42 day study and showed no sensitivity to time or temperature. Arsenic and Antimony have EPA MHTs of 180 days. Day 42 percentages remained at or near Day 0 percentages; this indicated a high amount of recovery. This study was not sufficient to determine the effect of exceeding the EPA MHT for Antimony or Arsenic. Mercury has an EPA MHT of 28 days; based on the EPA MHT guidelines, degradation could occur after 28 days. However, Mercury showed no degradation at 42 days. At Day 42, the recovery percentage for Mercury remained near Day 0 percentages at all three temperature

42

conditions for a SMHT of 42 days. There stability of the metals suggests there were no

known degradation components for metals within the tap water matrix.

VOC

The AIPH lab tested for 61 VOCs from various VOC categories. There were

seven in common with Maskarinec, *et al.,* (23) and eight in common with Love, *et al.,*

(22). The study of VOCs was conducted over a 42 day period and the results of 10 of the

61 VOCs are presented in Table 3 and Graphs 13 – 32 in Appendix A. The 10 VOCs

presented are Trihalomethanes (THM) and Solvents. According to the USGS, THMs and

solvents are the most frequent VOCs found in aquifers (38). The THMs presented are

Chloroform, Dibromochloromethane, Bromoform, and Bromodichloromethane. Results

from THM analysis show that Bromoform (Figure 10) had very similar degradation

patterns as Dibromochloromethane and Bromodichloromethane.
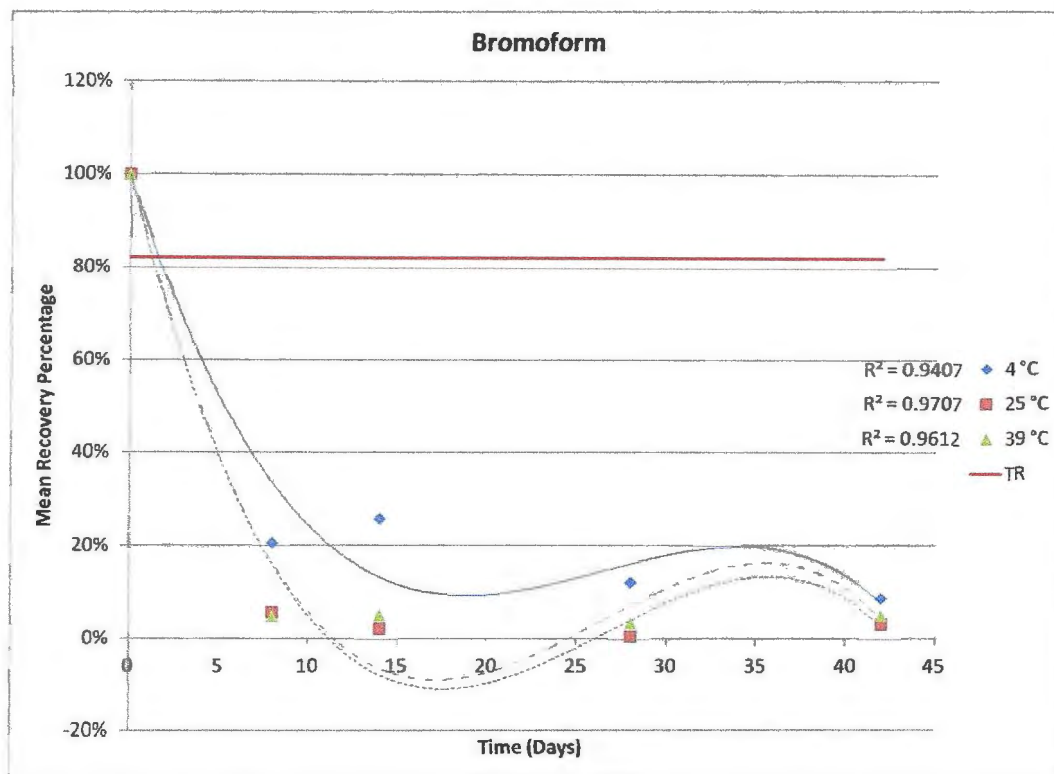
Figure 10: Graph of Bromoform stability results over 42 day study period.

Bromoform and the other THMs displayed exponential degradation patterns and had SMHTs of < 5 days at all three temperature conditions. The higher temperatures had SMHTs < 3 days. On Day 28 at 25 °C Bromoform had several non-detection results which proved difficult to determine any type of stability. The non-detection results were replaced with the method detection limit of 0.30 µg/L to determine the stability of Bromoform. The SMHT was determined to be consistent with Dibromochloromethane and Bromodichloromethane. The rapid degradation of the brominated THMs may be due hydrolysis. Bromine is larger atom than chlorine and less electronegative which may led to the rapid degradation.

Reviewing the effect time had on the stability of Chloroform; at the recommended temperature Chloroform had mean recovery percentages all above the *TR* (see Figure 11).

There were only three individual data points at the *TR*, these occurred on Days 14 and 42. The SMHT was determined to be 42 days at 4°C. At the elevated temperature conditions the degradation is inconsistent. At 25°C the mean recovery percentage increases between Days 14 and 28 and continues to rise at Day 42. The Day 8 and 14 mean recovery data points are below the *TR*. At 39°C there is an initial reduction in mean recovery percentage from Day 0 to Day 8, but after Day 8 there is a consistent increase in mean recovery concentration through Day 42. The Day 8 and 14 mean recovery data points are below the *TR*. Based on the results of this study, the SMHT is not able to be determined for the elevated temperatures for Chloroform as the stability was inconsistent.
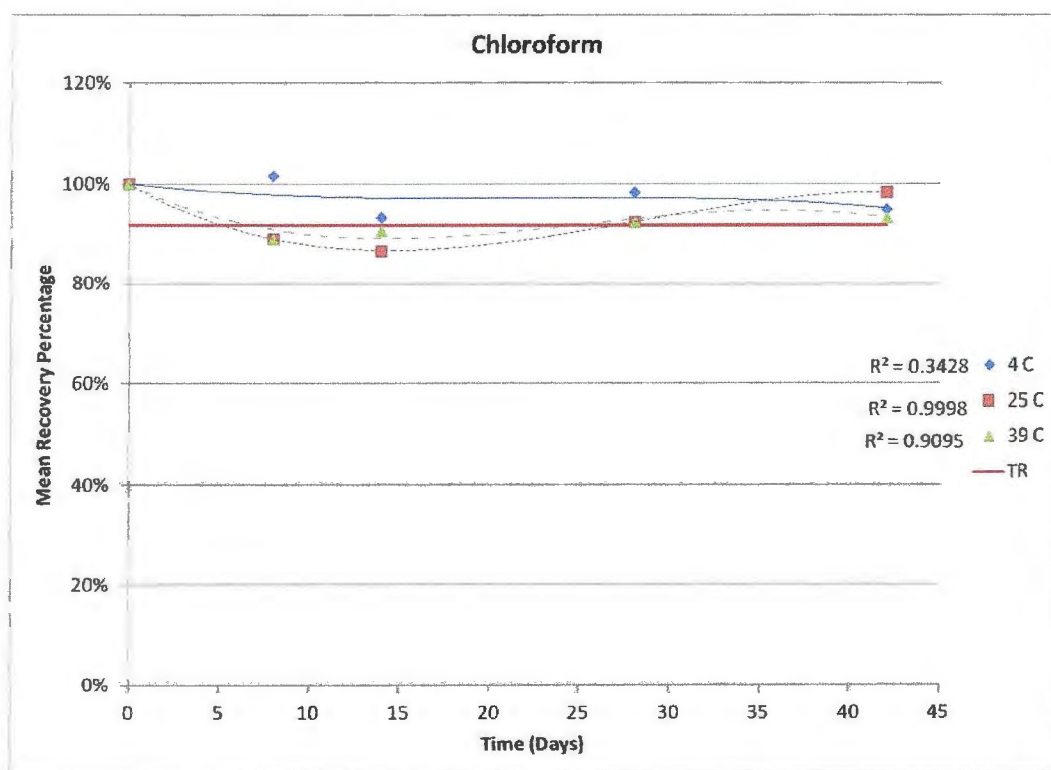
Figure 11: Graph of Chloroform stability results over 42 day study period.

Benzene and Toluene both showed consistent degradation at 4°C. Benzene was less stable with mean recovery concentration at the *TR* for Days 14 and 28. On Days 14 and 28 there was one individual data point below the *TR* compared to Day 42 that had all three individual data points at or below the *TR* (see Figure 12).
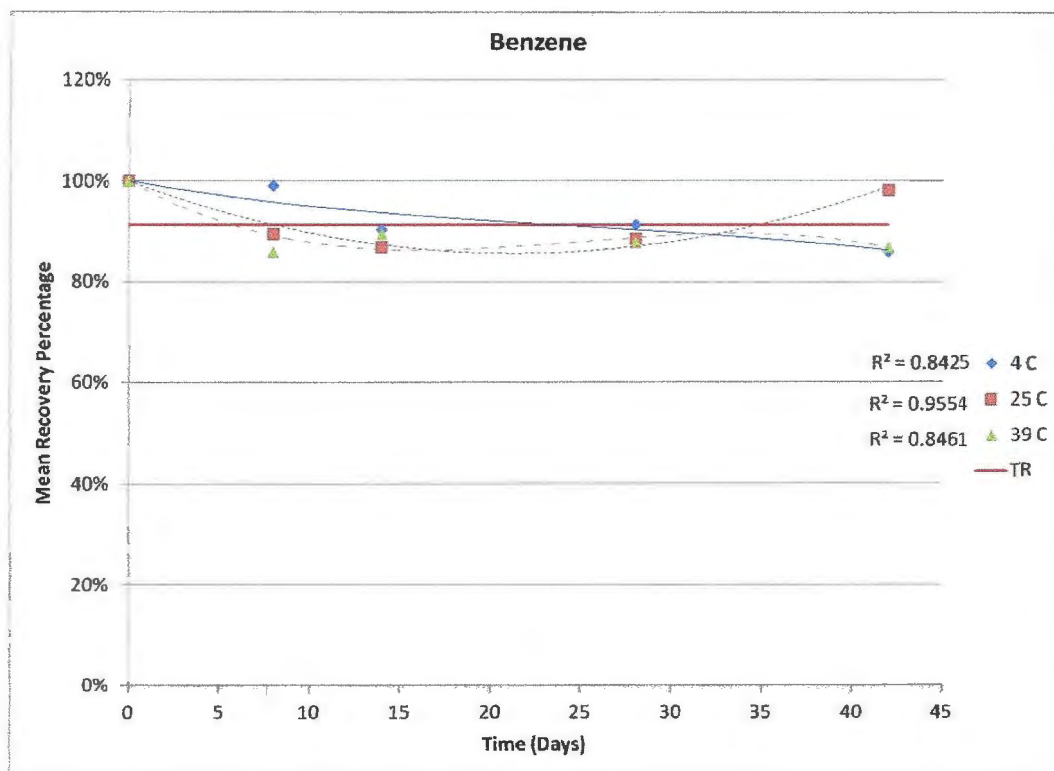
Figure 12: Graph of Benzene stability results over 42 day study period.

Toluene had mean recovery percentages above the *TR* throughout the study. It appears that exceeding time guideline alone did not hinder the stability of either analyte. At 4°C, the SMHT for Benzene was determined to be greater than 28 days but less than 42 days and the SMHT for Toluene was determined to be at least 42 days. Benzene and Toluene also showed consistent degradation at the elevated temperatures until Day 42 at 25°C where the mean recovery percentage rises above the *TR* for each analyte. At 39°C

the mean recovery percentage remains below the *TR* throughout the study. Exceeding the recommended temperature alone reduces the SMHT for Benzene to less than seven days and Toluene to greater than seven days but less than 14 days when at 25°C. At 39°C, both SMHTs were determined to be less than seven days. The reduction in stability as the temperature increased for Benzene and Toluene may be linked to its volatility.

The SMHTs for the solvents decreased as the temperatures increased. At 4°C, 1,1,1-Trichloroethane, Chloromethane and TCE all displayed similar degradation patterns (see Figure 13).
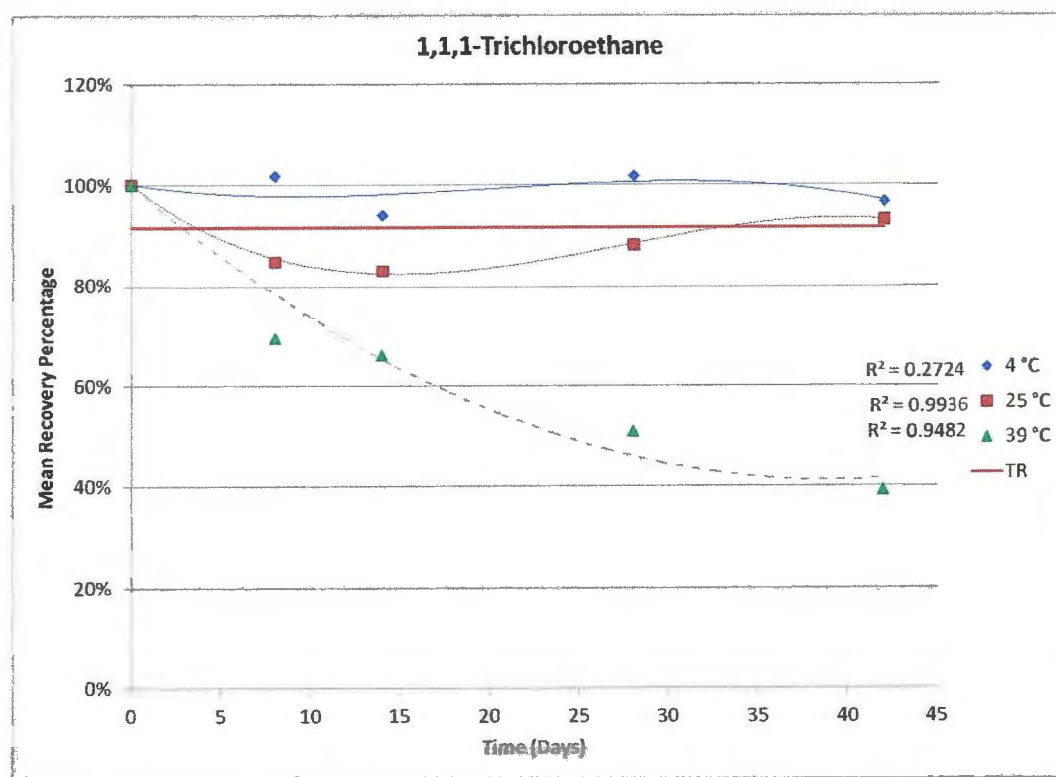
Figure 13: Graph of 1,1,1-Trichloroethane stability results over 42 day study period.

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Each analyte had mean recovery percentages above the *TR* and did not appear to be effected when exceeding the MHT alone. The mean recovery percentage for PCE at Days 14, 28, and 42 were all below the *TR*. The SMHT for 1,1,1-Trichloroethane, Chloromethane and TCE was determined to be at least 42 days and the SMHT for PCE was determined to be at least eight days. Exceeding the EPA MHT alone did not appear to inhibit the stability of 1,1,1-Trichloroethane, Chloromethane and TCE . At the elevated temperature conditions all mean recovery percentages were below the TR for each analyte except for 1,1,1-Trichloroethane and Chloromethane at 25°C. The SMHT for 1,1,1-Trichloroethane, Chloromethane, PCE, and TCE is less than eight days when exceeding the recommended temperature alone and the combination of time and temperature together. The chemical stability of 1,1,1-Trichloroethane, Chloromethane, TCE, Benzene, Toluene, and Chloroform does not appear to be hindered by time alone; each of the analytes had SMHTs above the 14 day guideline. The chemical stability of all THMs and solvents do appear to be hindered by temperature alone and the combination of time and temperature. Each analyte had SMHTs below the 14 day guideline.

The chemical stability of the VOCs appears to be greatly affected by temperature. VOCs have a high vapor pressure which causes them to rapidly volatilize. The evaporation rate increases as the increase in temperature increases the vapor pressure. At elevated temperatures the recovery percentage of VOC analyte would decrease proportional to the increase in temperature.

SVOC

The AIPH lab tests for 24 Semi-Volatile Organic Compounds, five phthalates are presented in Table 3 and Graphs 33 - 42 in Appendix A. During OIF and OEF the primary means for drinking water was bottled water. There continues to be concerns about phthalates migrating from water bottles into the water itself (13). Each of the phthalates had mean recovery percentages above the *TR* at each of the three temperature conditions. The phthalates as a group showed consistent stability, Figure 14 shows the stability of Di-n-butylphthalate. Di-n-butylphthalate had a high RSD which resulted in a *TR* less than 60%. The 80% recovery is significant and may represent degradation, but it is unsure since the Day 0 RSD is high and reduced the confidence.
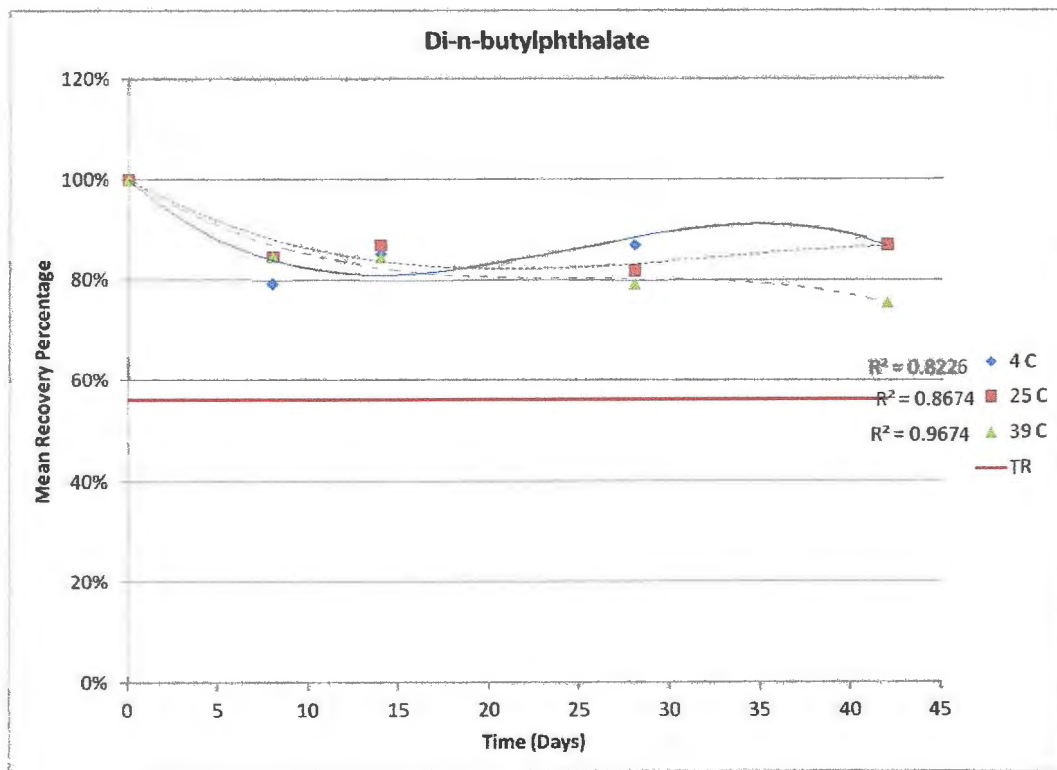
Figure 14: Graph of Di-n-butylphthalate stability results over 42 day study period.

The SMHT for each phthalate was determined to be 42 days at each temperature condition. Exceeding the EPA MHT did not appear to inhibit the stability of the phthalates. At 4°C each phthalate had a SMHT of 42 days. Each of the elevated temperatures was below the boiling point for each of the phthalates; the SMHT remained at 42 days. The combined effect of exceeding the EPA MHT and recommended temperature did not produce any degradation below the *TR* for either phthalate studied. Volatilization is not a dominate fate process for SVOCs due to their low Henry's Law constant and vapor pressure.

CYANIDE

The Cyanide (CN) stability study was conducted over a 31 day period, the results are presented in Table 3 and Graphs 43 – 46 in Appendix A. The first iteration of the CN study used sodium hydroxide (NaOH) as a preservative and tap water as the matrix. All samples had non-detect results on Day 0, which made it impossible to determine the SMHT or the temperature effects on CN stability in drinking water. The lab tap water contained 0.8 ppm of free available chlorine, a known interfering constituent based on the OIA 1677 method of analysis. It is believed that the non-detects are from chlorine interferences in the tap water (5). Chlorinated tap water would not be a good choice for future research without first analyzing the tap water to determine the presence of interfering constituents. Any research using tap water should plan to address the interfering constituents.

Due to the non-detects from the lab tap water, it was decided to expand this portion of the study using de-ionized (DI) water and untreated water from a private

household well while continuing to use NaOH as the preservative to control for the possible chlorine confounder. The chemical stability for CN in untreated well water remained above the *TR* at each analysis point for 4°C and 25°C. At 39°C the mean recovery percentage remained above the *TR* until Day 28. The chemical stability dropped below the *TR* between Days 21 and 28. The chemical stability for CN in DI water at 4°C and 25°C was similar to that of CN in well water at the same temperatures. At 39°C the mean recovery percentage fell below the *TR* between Days 7 and 14. Exceeding the EPA MHT alone did not produce any degradation below the *TR* for CN in either water matrix at 4°C; all mean recovery percentages were above the *TR*.

Exceeding the recommended temperature did not produce degradation below the *TR* at 25°C for either water matrix; all mean recovery percentages were above the *TRs*. At 39°C in DI water the mean recovery percentage fell below the *TR* at Day 14 with day seven above the *TR* (see Figure 15). At 39°C in well water the Day 28 mean recovery percentage was below the *TR* with one data point being a possible outlier. The SMHT for CN in both matrices at 4°C and 25°C was determined to be 42 days. The SMHT for CN in DI water was greater than seven days but less than 14 days, for CN in well water the SMHT was greater than 21 days but less than 28 days.
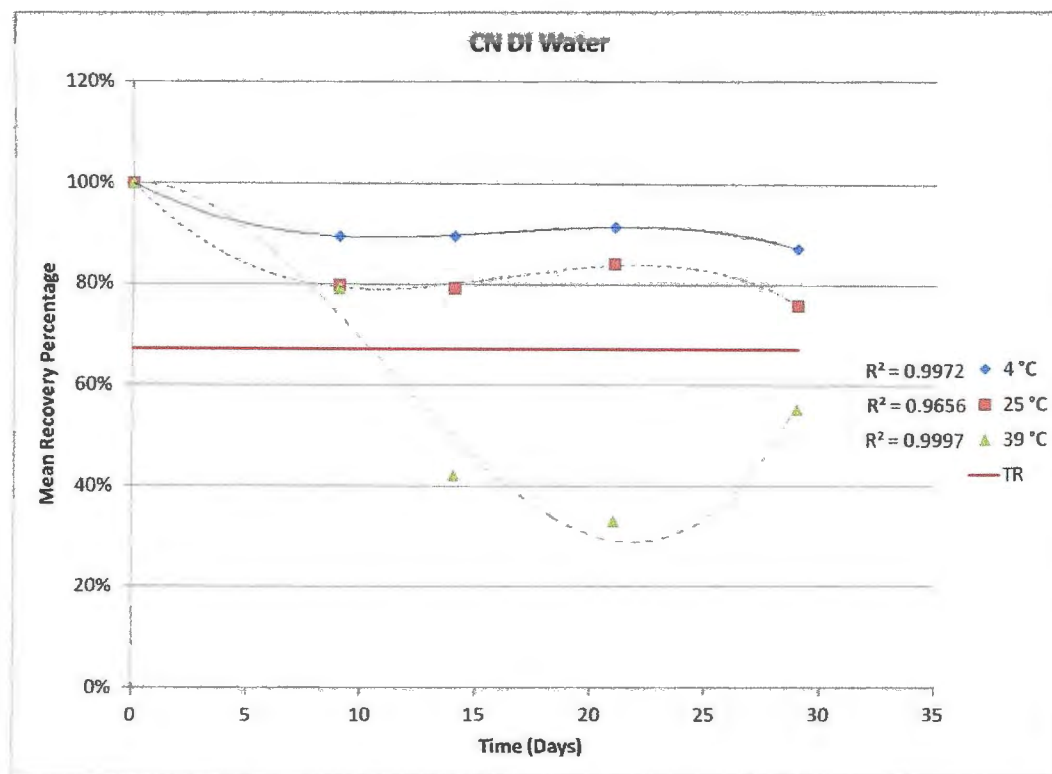
Figure 15: Graph of CN in DI water stability results over 31 day study period.

CYANIDE PRESERVATIVE STUDY

The AIPH lab uses NaOH as the preferred preservative for CN based on ASTM D7365; however, the OIA 1677 method of analysis states that NaOH is best suited for pH control (5) and ascorbic acid should be used as the preservative when chlorine is suspected (3). Due to the non-detect results from the tap water matrix, a preservative study was conducted to compare the recovery using NaOH and Ascorbic acid as preservatives at 4°C to control for chlorine, Appendix A, Graphs 47 and 48. The preservative study used DI water and tap water with three preservative conditions; no preservative, ascorbic acid only, and ascorbic acid with NaOH. The results of the preservative study showed that ascorbic acid may not be a sufficient preservative when samples can't be analyzed within 24 hours per ASTM D7365. When comparing the CN

in DI water graphs (Figures 43 and 44) from the stability study which used NaOH only as the preservative to CN in DI water for the preservative study which used ascorbic acid only and ascorbic acid with NaOH as preservatives; the ascorbic acid preservative produced non-detect results when used alone and with NaOH (see Figure 16). In Figure 16, No Pres is no preservative added, AA is ascorbic acid only, and AA/NaOH is ascorbic acid with sodium hydroxide preservative mix.
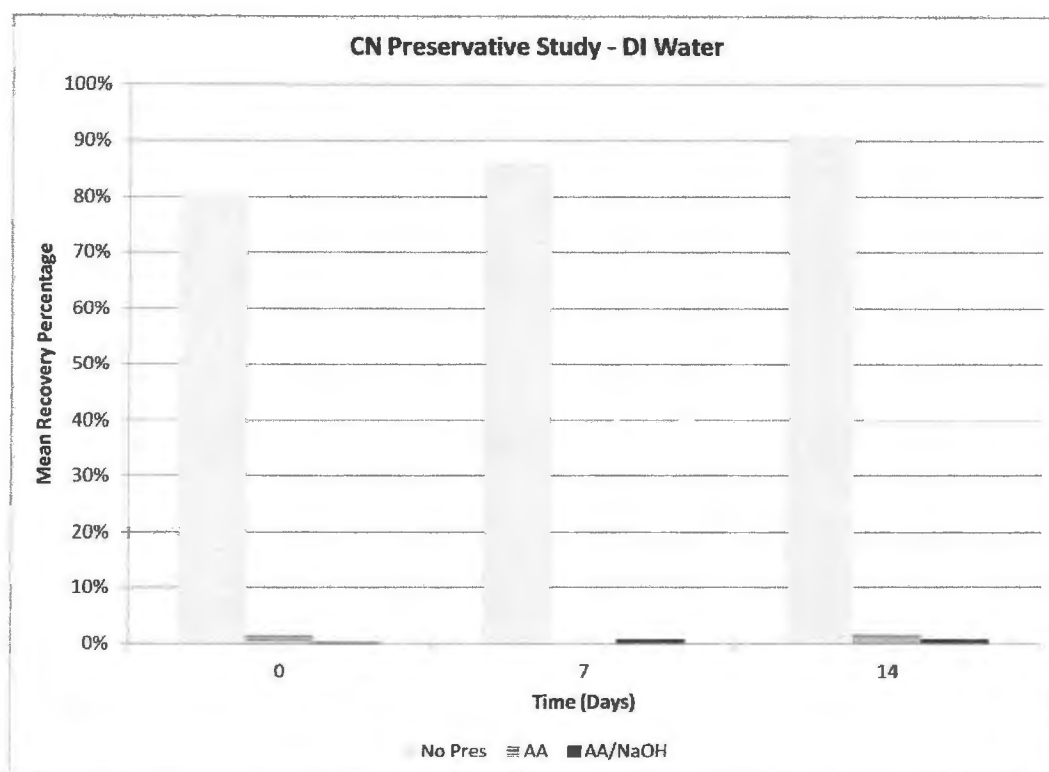
Figure 16: Bar graph of CN in DI water from preservative study.

SUMMARY

The average AIPH receipt temperature for drinking water kits was 19 °C during 2011 and the samples likely experienced higher temperatures in-transit during summer

months.   This study demonstrated that several analyst remained chemically stable beyond the EPA MHT as evidenced by the SMHT being greater that the EPA MHT. Matrix effects will need to be addressed to account for some interference with the analytes. The longest the chemical stability could be determined based on this study is a hold time equal to the duration of the analyte study period. There is not enough data to be predictive beyond this point. This study also went a step further to investigate the temperature influence on these analytes in addition to exceeding the EPA MHTs. In some cases it was difficult to apply the ASTM D4841 method of determining the MHT due to inconsistent degradation and some mean recovery percentages increasing after previous recoveries were lower. The fluctuation in mean recovery percentage may be attributed to the difference in QC for each day of analysis, as in the case for Endothall where the QC results varied by 10 – 40%. In the cases where it was difficult to apply the ASTM method, a range of possible stability was provided. The findings show temperature does have a major influence on some analytes and may influence others.

Chapter 5 - Conclusion

The intent of this study was to determine the effect exceeding the EPA MHT and storage temperature had on drinking water samples from forward deployed units by evaluating the stability of different analytes at elevated temperatures at and beyond the EPA MHT. The findings are summarized below addressing the specific aims used to answer the hypothesis.

1. *Analyze contaminant-spiked water samples from a tap water matrix to measure the effect of exceeding the EPA-recommended MHT for the analytes of interest.*

From this study there were 23 analytes evaluated in this study, 19 of 23 (83%) of the analytes held at 4°C were chemically stable at or beyond the EPA MHT. The four analytes not chemically stable were Tetrachloroethene, Bromoform, Bromodichloromethane, and Dibromochloromethane. The lack of stability for Tetrachloroethene may be due to it being heavier than water and the amount that does not volatize sinks in the water away from water surface (1). However, the most likely cause for the instability of Tetrachloroethene, Bromoform, Bromodichloromethane, and Dibromochloromethane may be due to volalitization and an unknown constituent in the tap water. There were four Trihalomethanes evaluated; Bromoform, Bromodichloromethane, Dibromochloromethane, and Chloroform. Chloroform was the only Trihalomethane chemically stable at and beyond the EPA MHT when stored at 4°C; its chemical stability appeared to be at least 42 days compared to less than 5 days for the other three at 4°C. Exceeding the time guidance had little effect on the chemical stability of the remaining selected analytes despite using a tap water matrix.

2. *Analyze contaminant-spiked water samples from a tap water matrix to measure the effect of exceeding the EPA-recommended temperature of 4 °C for the analytes of interest.*

There were 13 of 23 (56%) analytes stable at their EPA MHT when held at 25 °C. The unstable analytes were VOC – THMs and VOC - Solvents. At 39 °C, 12 of 23 (52%) analytes were stable at or beyond the EPA MHT. The unstable analytes were VOC – THMs, VOC – Solvents, and CN in DI water (see Table 3). Exceeding the EPA storage temperature had a greater effect on the stability of the VOCs whose stability decreased as the temperature increased. Based on the high vapor pressure of VOCs, temperature is expected to have a greater effect on the chemical stability of the analytes. It is important to properly manage the temperature of samples when stored and while in-transit when sampling for VOCs. The current method of cold chain management is inadequate to maintain samples at 4°C, but may be able to maintain samples at 25°C. Reviewing the 224 sample receipt data, 90% of the sampling kits were received at 25°C or less. This does not account for the possible in-transit temperature profile which may have exposed sampling kits to higher temperatures for an extended period of time.

3. *Investigate the combined effects of exceeding the EPA recommended MHT and shipping temperature.*

When samples were held beyond their MHT and at elevated temperatures 13 of 23 (56%) analytes appeared to be stable at 25°C, 12 of 23 (52%) analytes appeared to be stable at 39 °C. Outside of metals whose EPA MHT is 28 days for Mercury and 180 days for Antimony and Arsenic, the most stable were the herbicides and phthalates. Chloroform appeared to be the most stable of the VOCs. Visually evaluating the graphs,

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exceeding the EPA MHT and storage temperature produced less chemical stability than exceeding the EPA MHT or storage temperature alone, but was temperature dependent.

4. *Conduct preservation study for Cyanide using chlorinated tap water and pure de-ionized water comparing samples with no preservative, ascorbic acid only, and ascorbic acid and sodium hydroxide to determine the extent of chlorine as an interfering contaminant.*

The Cyanide preservative study determined that ascorbic acid was not a suitable preservative when samples cannot be analyzed within 24 hours per ASTM D7365. Cyanide samples are analyzed by an outside lab contracted by AIPH. The CN samples are analyzed the one day after receipt by the contract lab. At each analysis point for CN in DI water, ascorbic acid produced non-detect results. When using NaOH alone as in the stability study, the CN ion was recovered in DI water and untreated well water.

SIGNIFICANCE

The US military could use data from this line of research to develop military specific shipping and storage guidelines for environmental water samples. The current process of DESP accepting samples for analysis may not have a negative impact on sample analysis for samples held at 4°C. In reference to cold chain management, the current method may be sufficient if samples can be received within a certain time. During the early stages of military operations when logistic supply and transport systems are immature, environmental samples could be shipped to the US Army Public Health Command Region - Europe analytical lab for analysis. This research could also lead to a change in military deployment doctrine by providing justification to deploy analytical

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capabilities closer to the point of collection to reduce the time and distance samples will need to travel for analyses. It would also reduce the requirement for cold chain management of environmental samples.

The results of the line of research could also add to the work previously conducted by adding valuable temperature effects for single storage temperatures and for varying temperature profiles. Other environmental labs could use this data to develop shipping and storage guidelines for their labs. The EPA could use the results to one day modify their guidelines as well.

FUTURE RESEARCH

The research presented here is a first attempt to characterize the impact of exceeding EPA-recommended guidelines for holding times and maximum storage temperatures on water samples collected in military forward deployed settings. The data will likely provide a solid foundation for USAPHC to use and build upon in developing standards and guidelines for environmental water samples collected in overseas contingency operations. Future research along this line is listed below:

1. As samples are transported from the point of collection to the environmental lab over long distances, they may be subjected to a wide range of temperatures during travel. The effect of this temperature profile on the recovery concentration is unknown. To further this preliminary research, it is recommended that future research is conducted to determine the effect a temperature profile will have on environmental water samples. By using temperature data loggers that can record temperatures at a set interval over a period of weeks, a temperature profile could be developed to study the effect

that varying temperatures would have on the recovery concentration and stability.

2. Studying the sample collection technique to determine the optimum vial fill rate and fill technique (side pour, top pour, or bottom fill) could provide useful information to improve the quality of recovery concentration of samples collected in the field.

3. Using the receipt conditions and concentrations to determine the actual contaminant concentration level at the point of collection based on a known degradation rate would allow for determination of the actual water contaminant concentration levels at austere base locations.

LIMITATIONS

This research evaluated the effect on chemical stability when the EPA MHT and storage temperature for water samples in a complex water matrix were exceeded. While useful information was derived from this study, there were limitations. The major limitation was the use of ASTM D4841, which is matrix specific. This study used AIPH lab tap water which would have a different characteristic from other water sources. At best, correlations may be made based on similar water matrices. This leads into the next limitation of not characterizing the tap water. It was difficult to discuss unexpected results from possible interfering constituents. Some methods provided a list of known chemical interferences and procedures to mitigate their effects. Not taking steps to mitigate known interferences could lead to false conclusions. Another significant limitation was the lack of enough data analysis points. It was difficult to develop best-fit curves to use in determining the MHT because there were only a few data points to draw

from and the degradation patterns were not consistent. Also the curves used in the study were 2nd or 3rd degree polynomials which made it difficult to determine chemical stability. Using statistical methods to determine if individual data points were outliers was also challenging because the sample size at a specific analysis point was too small. The confidence of the best-fit curves was reduced by using three initial replicate samples as opposed to the 10 recommended by ASTM D4841.

REFERENCES

1. Agency for Toxic Substances and Disease Registry. 1997. Toxicological Profile for Trichloroethylene. Department Health and Human Services-Public Health Service,

2. American Society for Testing Materials. 1988 (2008). Standard Practice for Estimation of Holding Time for Water Samples Containing Organic and Inorganic Constituents D4841-88.

3. American Society for Testing Materials. 2009. Standard Practice for Sampling, Preservation and Mitigating Interferences in Water Samples for Analysis of Cyanide D7365 − 09a.

4. American Society for Testing Materials. 2009. Standard Test Methods for Cyanides in Water D2036-09.

5. Capital Controls. 2002. Elimination of Cyanide Compounds with Chlorine. Severn Trent Purification Inc,

6. Craig S. 2010. History of Public Health. Bethesda: Uniformed Services University of the Health Sciences

7. Environmental Protection Agency. 2007. Phthalates. U.S. EPA Toxicity and Exposure Assessment for Children's Health,

8. Environmental Protection Agency. 2013. Code of Federal Regulations 40 CFR Part 141.

9. Food and Agriculture Organization of the United Nations. 2008. Specifications and Evaluations for Agricultural Pesticides. Food and Agricultural Organization of the United Nations,

10. Frank P, Comes R. 1967. Herbicidal Residues in Pond Water and Hydrosoil. *Weeds*:210 - 3

11. Gallotta FDC, Lourenço RA, Araújo LFMd. 2010. Evaluation of Holding Time for Polycyclic Aromatic Hydrocarbon (PAH) Analysis in Saline Water Samples. *Environmental Forensics* 11:309-14

12. Gellasch CA, Calix LC. 2007. Preventive Medicine Support in Afghanistan During Operation Enduring Freedom VI. *Army Medical Department Journal* April - June:56-64

13. Greifenstein M, White DW, Stubner A, Hout J, Whelton AJ. 2013. Impact of temperature and storage duration on the chemical and odor quality of military

packaged water in polyethylene terephthalate bottles. *Science of The Total Environment* 456–457:376-83

14. Grzenda AR, Nicholson HP, Cox WS. 1966. Persistence of four herbicides in pond water. *Journal (American Water Works Association)*:326-32

15. Heller JM. 2009. The Periodic Occupational and Environmental Monitoring Summary (POEMS)-History, Intent, and Relationship to Individual Exposures and Health Outcomes. US Army Public Health Command

16. Heller JM, Resta JJ. 2001. Occupational and Environmental Health Surveillance. *U.S. Army Medical Department Journal* October - December:36 - 43

17. Hiltibran RC. 1962. Duration of Toxicity of Endothall in Water. *Weeds* 10:17-9

18. Hiltibran RC, Underwood DL, Fickle JS. 1972. Fate of Diquat in the Aquatic Environment, University of Illinois

19. International Cyanide Management Institute. October 29 2012. *Cyanide Chemistry*. http://www.cyanidecode.org/cyanide_chemistry.php

20. Kirkpatrick JS, Moser C, Hutchens BE. 2006. Deployment Occupational and Environmental Health Surveillance: Enhancing the Warfighter's Force Health Protection and Readiness. *Army Medical Department Journal* April - June 2006:50-4

21. Langeland KA, Warner JP. 1986. Persistence of Diquat, Endothall, and Fluridone in Ponds. *Journal of Aquatic Plant Management* 24:43-6

22. Love JT, Delzer GC, Abner SR, Zogorski JS. 1999. *Study Design and Analytical Results Used to Evaluate Stability of Volatile Organic Compounds in Water Matrices*. U.S. Department of the Interior, U.S. Geological Survey

23. Maskarinec MP, Johnson LH, Holladay SK, Moody RL, Bayne CK, Jenkins RA. 1990. Stability of volatile organic compounds in environmental water samples during transport and storage. *Environmental Science & Technology* 24:1665-70

24. National Center for Medical Intelligence. March 8 2013. *Environmental Health Risk Assessment*. https://www.intelink.gov/ncmi/country.php?country=IRQ

25. Parker L, Britt S. 2012. The Effect of Bottle Fill Rate and Pour Technique on the Recovery of Volatile Organics. *Groundwater Monitoring & Remediation* 32:78-86

26. Paulson TL. 2008. *The Fate of Cyanide Ion in Aquatic Environment*. Rutgers University, Camden

27.   Schumacher B. August 24 2012. *Sample Holding Time Re-Evaluation.*
       http://www.epa.gov/esd/cmb/tasks/holding.htm

28.   Sikka HC, Rice CP. 1973. Persistence of endothall in aquatic environment as
       determined by gas-liquid chromatography. *Journal of Agricultural and Food
       Chemistry* 21:842-6

29.   Simsiman GV, Chesters G. 1975. Persistence of endothall in the aquatic
       environment. *Water Air Soil Pollut* 4:399-413

30.   Simsiman GV, Daniel TC, Chesters G. 1976. Diquate and Endothall: Their fates
       in the Environment. *Residue Reviews*:131-74

31.   United States Army, United States Navy, United States Air Force. 2010. Sanitary
       control and serveillance of field water supplies. In *TB MED 577/NAVMED P-
       5010-10/AFMAN 48-138_IP*: Departments of the Army Navy Air Force,

32.   US Army Corps of Engineers. 1988. Aquatic Plant Identification and Herbicide
       Use Guide. Vicksburg, MS: Department of the Army,

33.   US Army Public Health Command. 2010. Environmental Health Risk Assessment
       and Chemical Exposure Guidelines for Deployed Military Personnel. In *Technical
       Guide 230*: US Army Public Health Command,

34.   US Army Public Health Command. 2012. Army Institute of Publuc Health
       Laboratory Sciences Customer Service Manual. US Army Public Health
       Command,

35.   Van Belle G. 2011. *Statistical rules of thumb.* Wiley-Interscience

36.   Yeo R. 1967. Dissipation of diquat and paraquat, and effects on aquatic weeds
       and fish. *Weeds*:42-6

37.   Yeo RR. 1970. Dissipation of Endothall and Effects on Aquatic Weeds and Fish.
       Weed Science:282-4

38.   Zogorski JSC, Janet M.; Ivahnenko, Tamara; Lapham, Wayne W.; Moran,
       Michael J.; Rowe, Barbara L.; Squillace, Paul J.; Toccalino, Patricia L. 2006.
       Volatile Organic Compounds in the Nation's Ground Water and Drinking-Water
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Appendix A - Graphs with Best-Fit Curves

HERBICIDES
Endothall
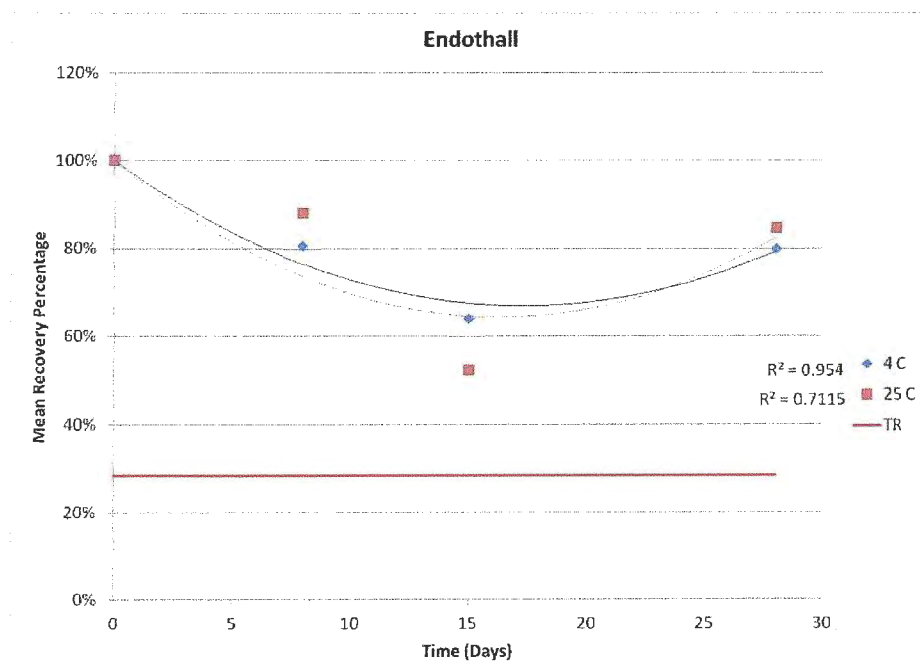
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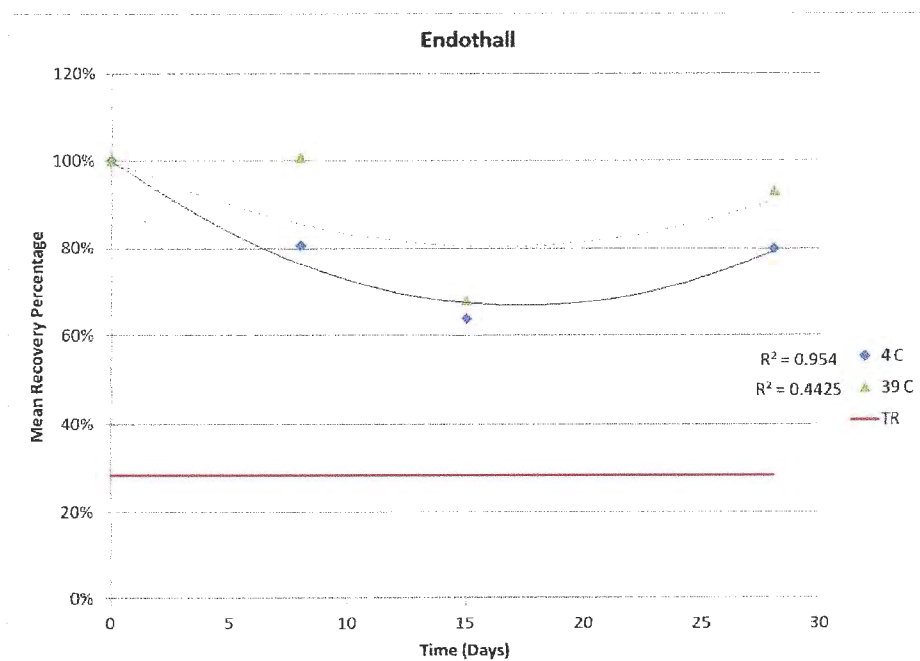
Graph #2

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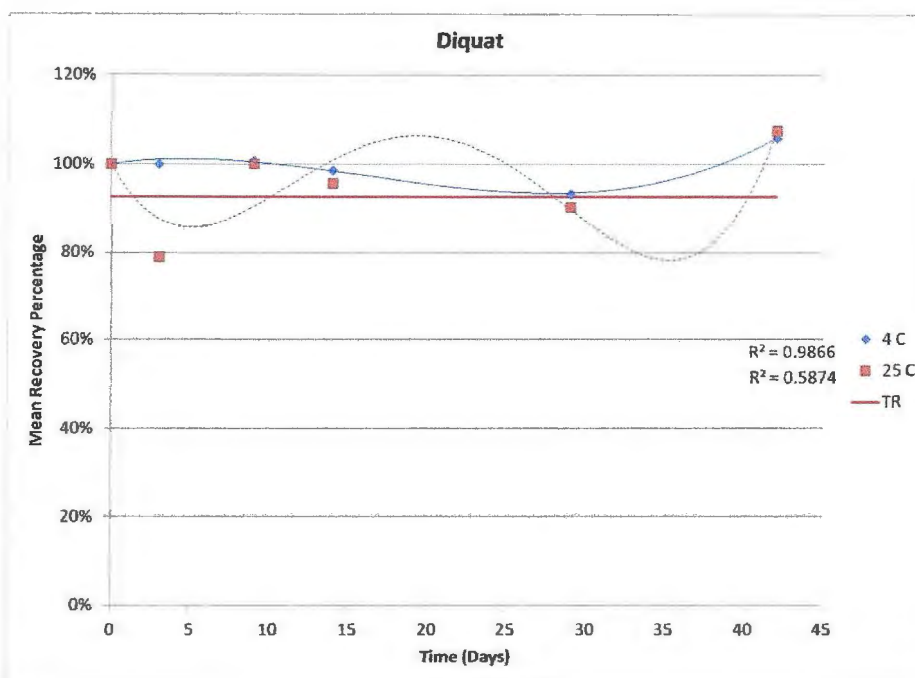
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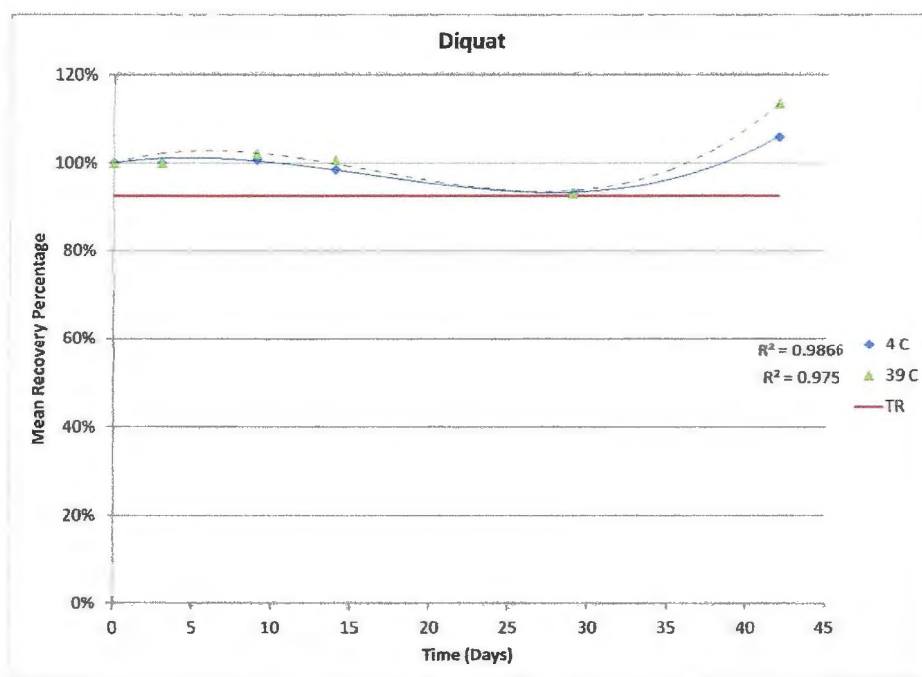
Graph #4

Paraquat
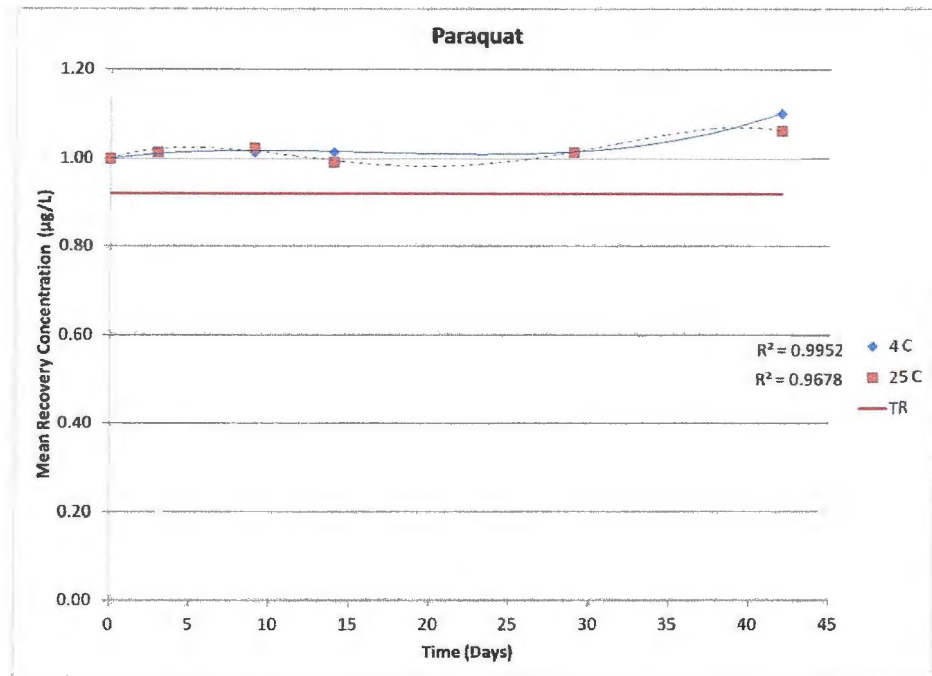
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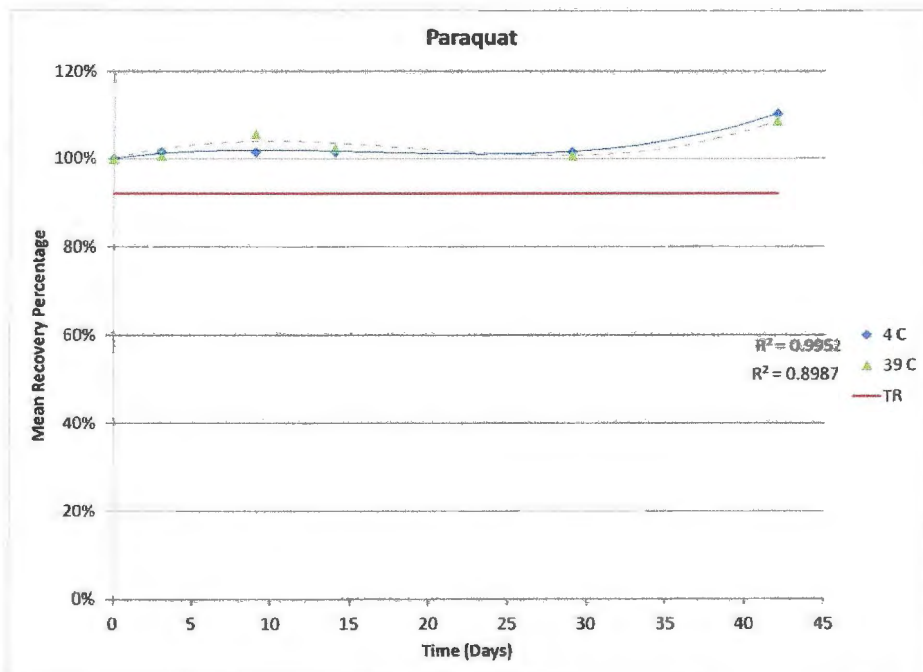
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METALS
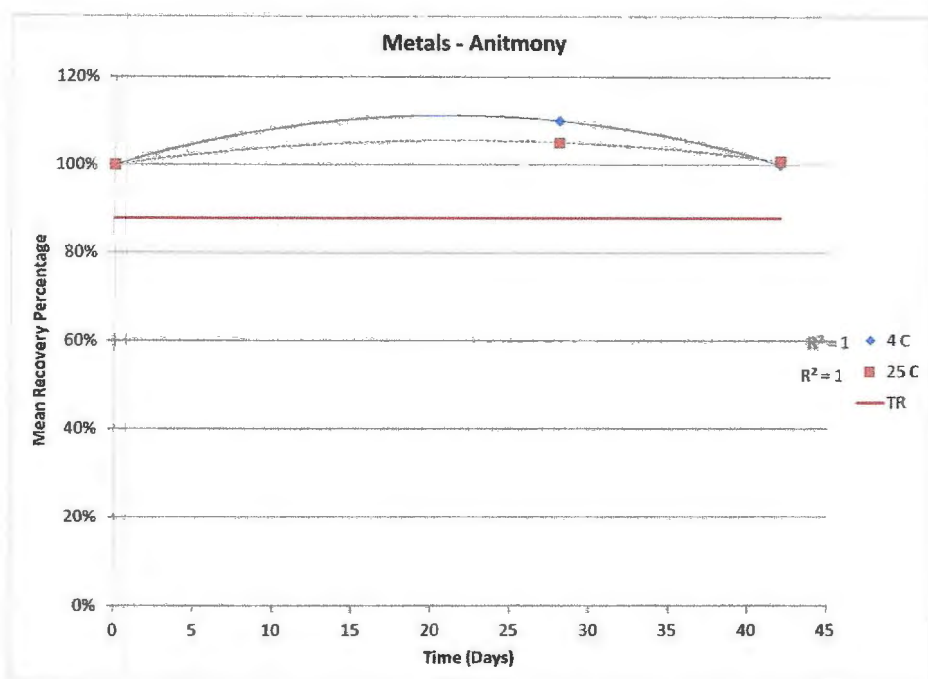
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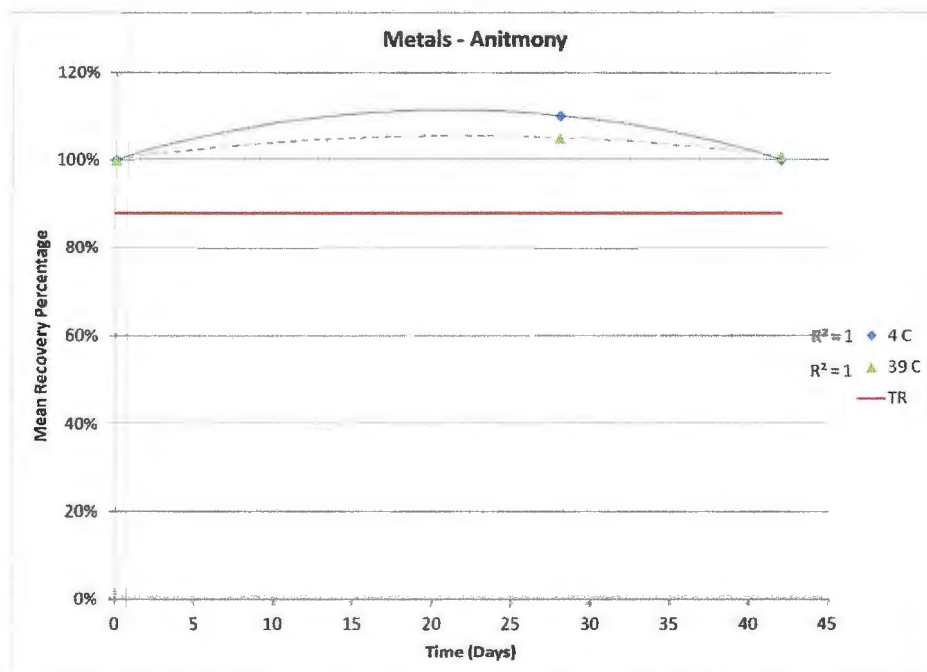
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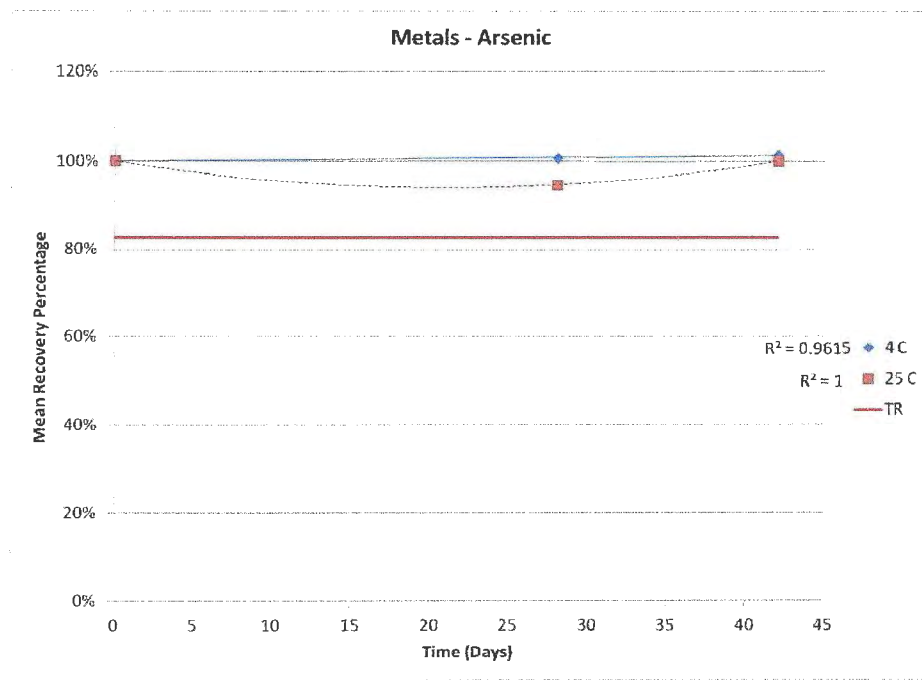
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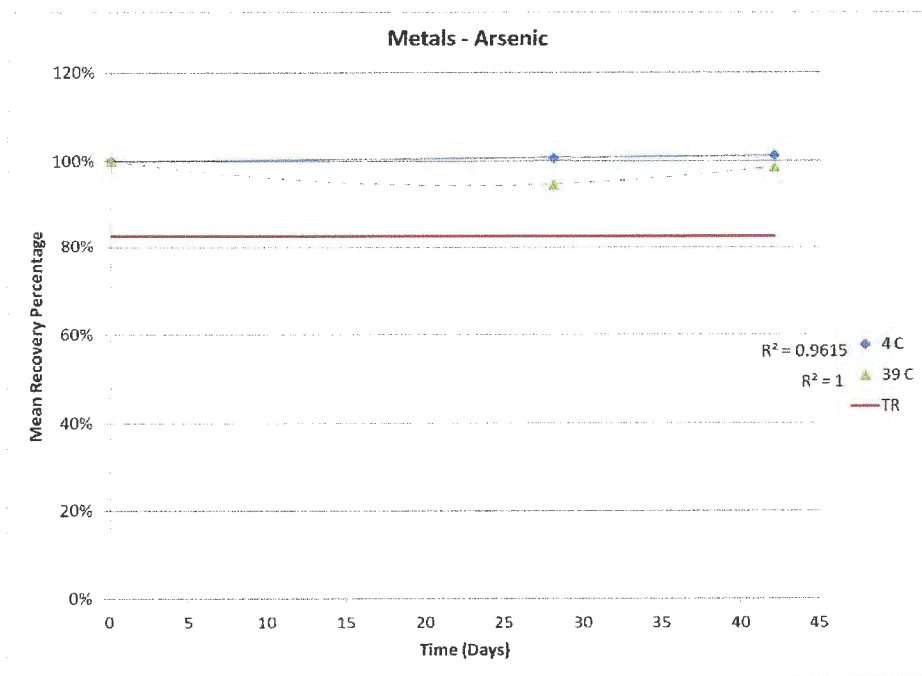
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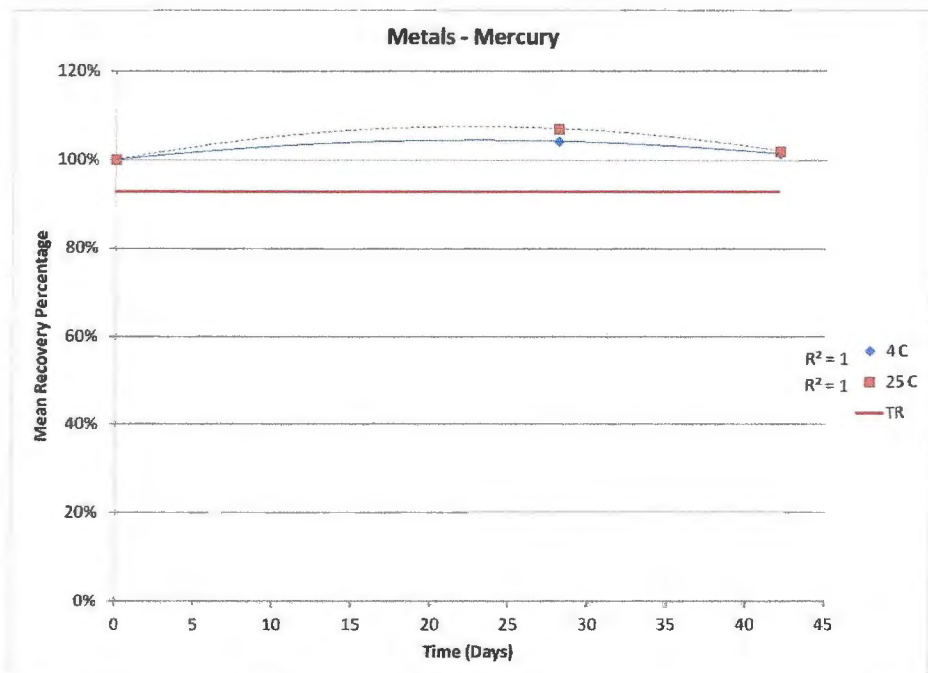
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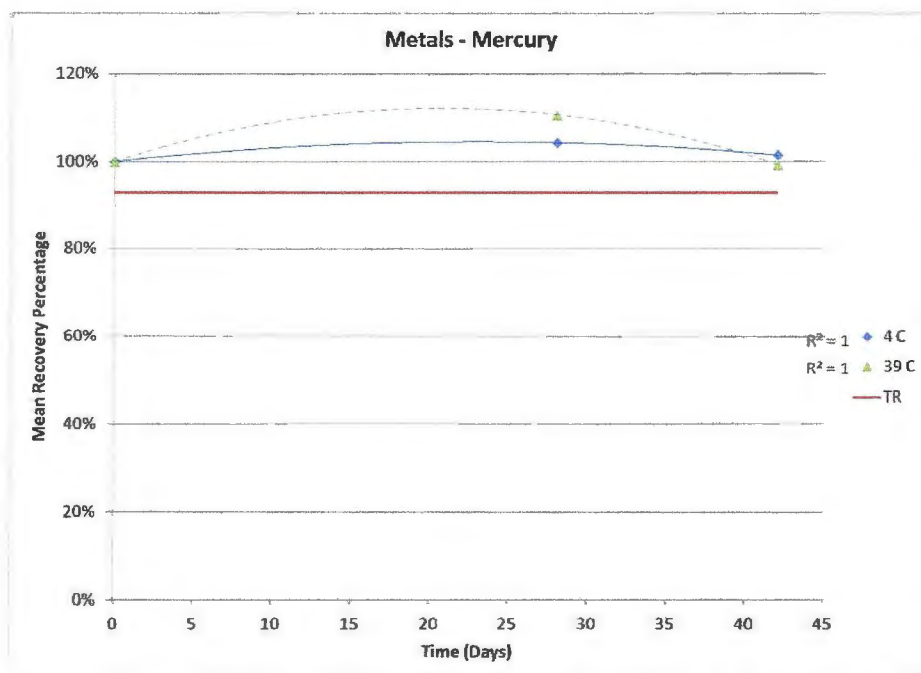
Graph #12

VOLATILE ORGANIC COMPOUNDS
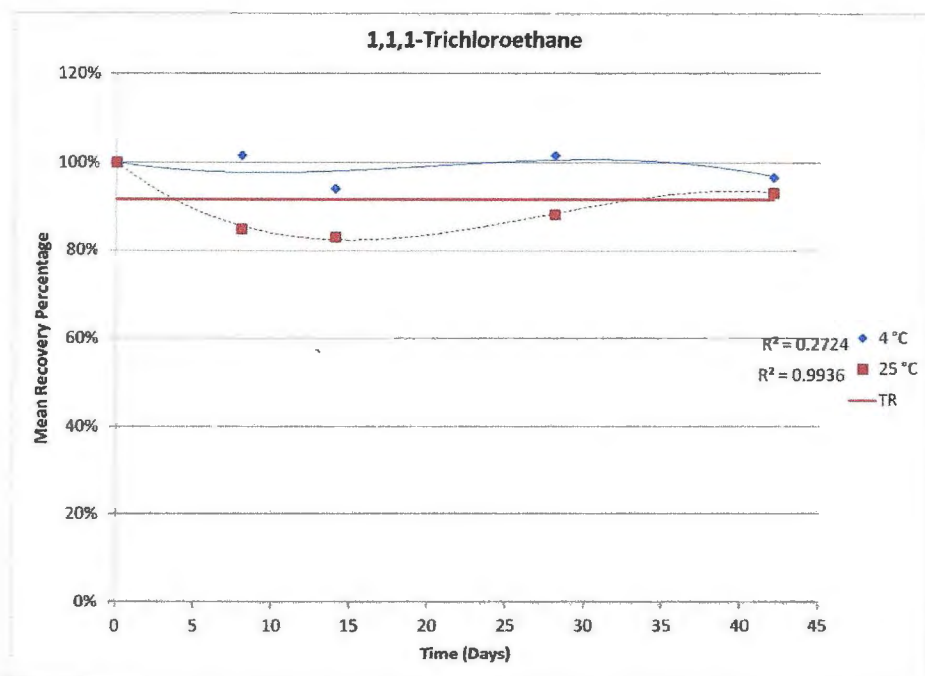
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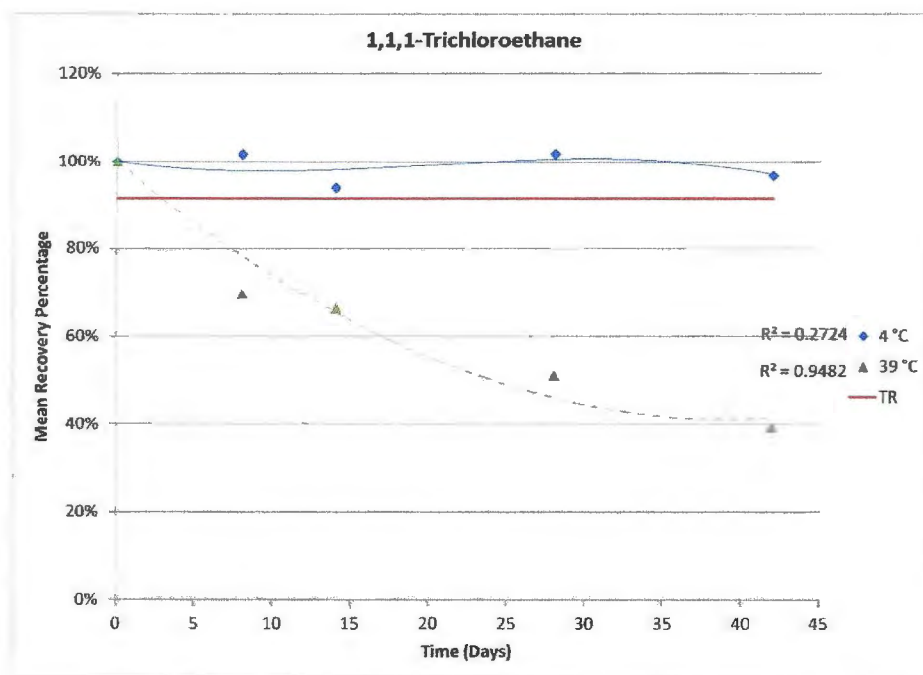
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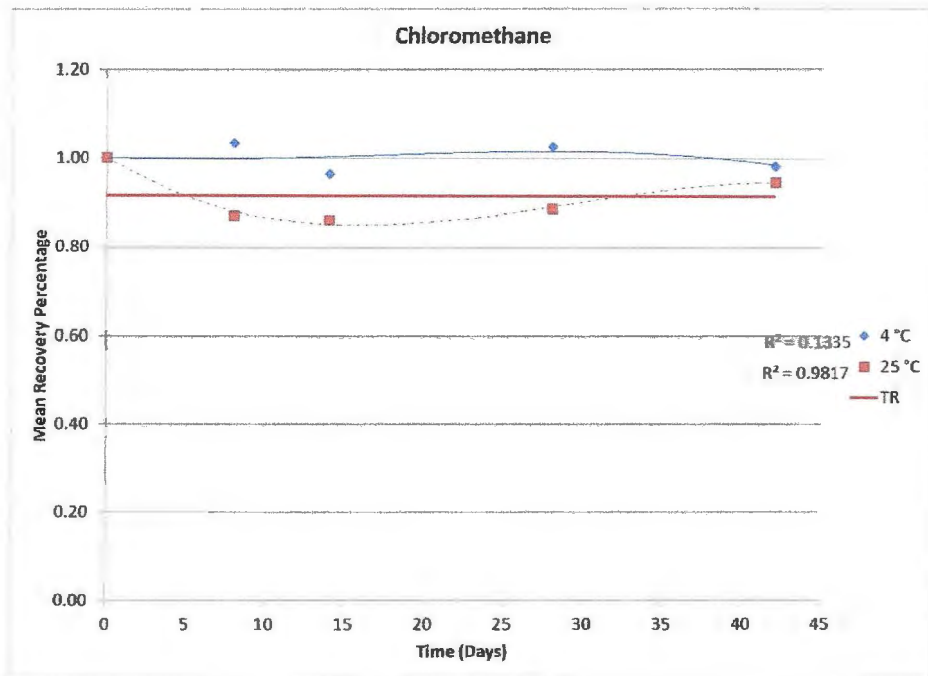
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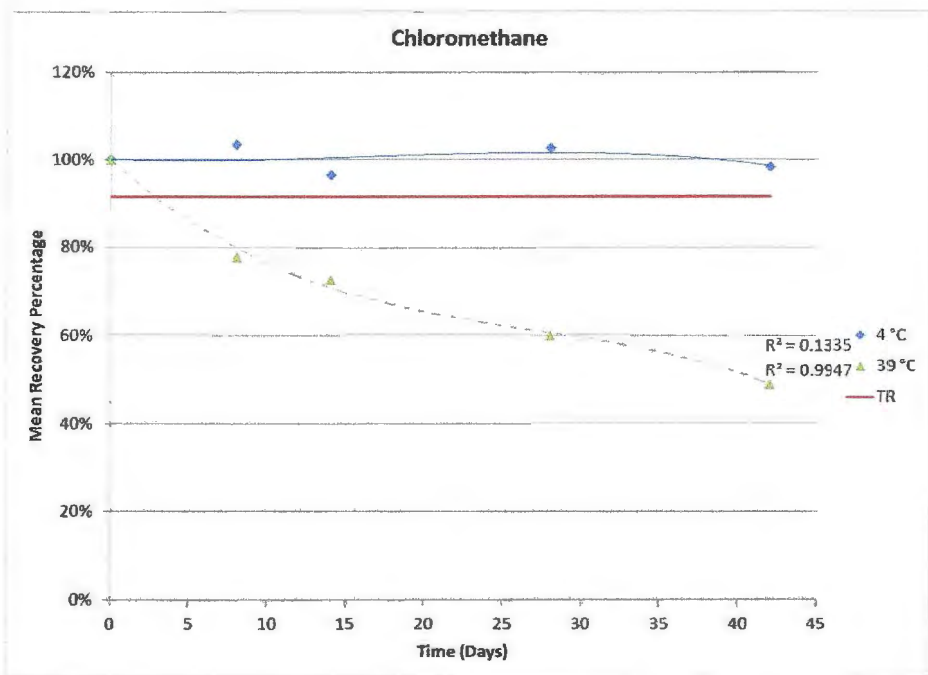
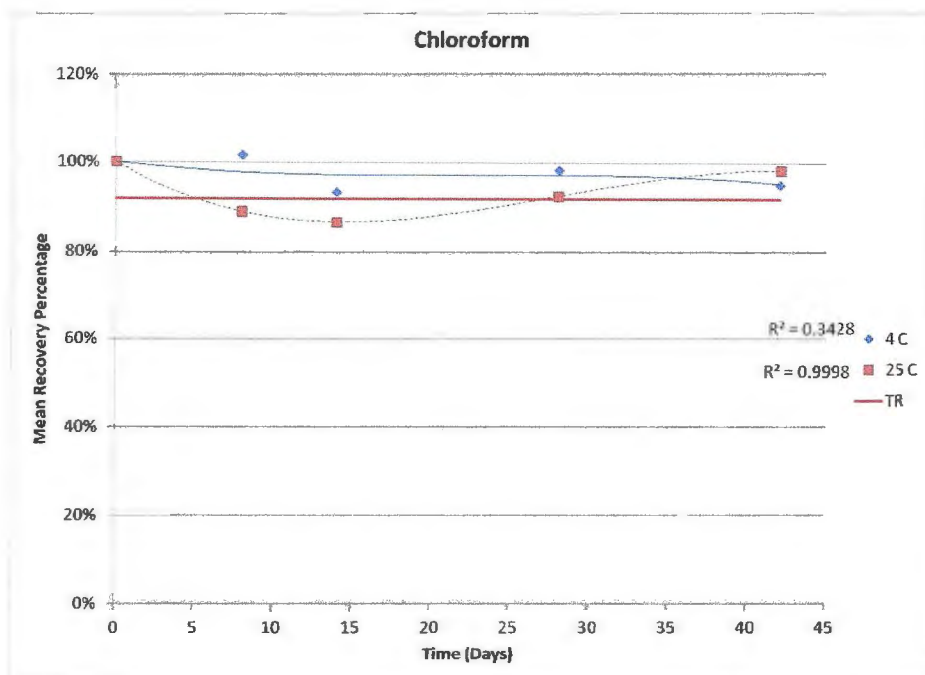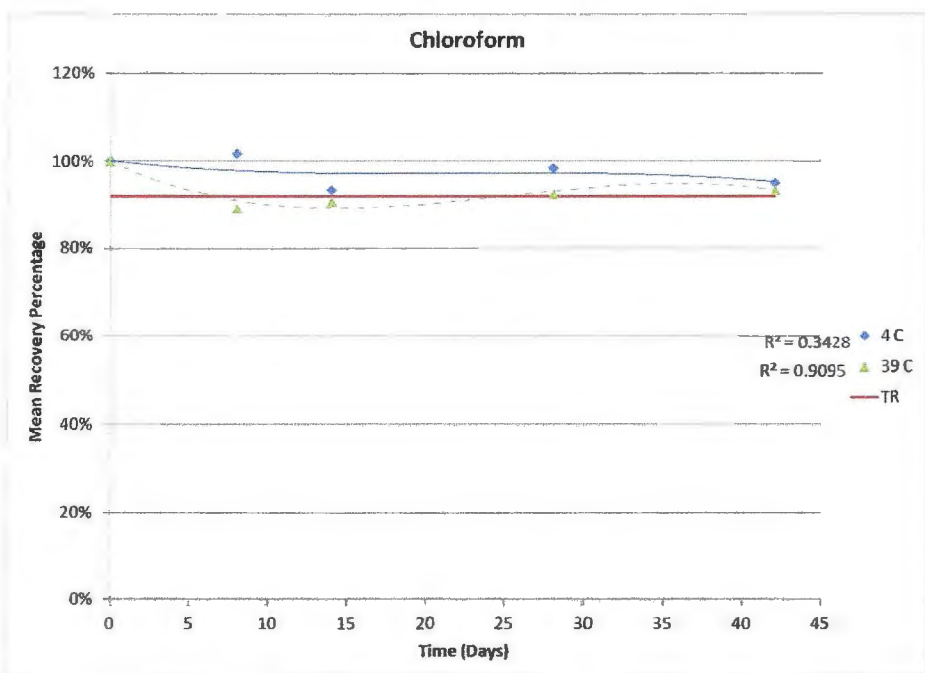

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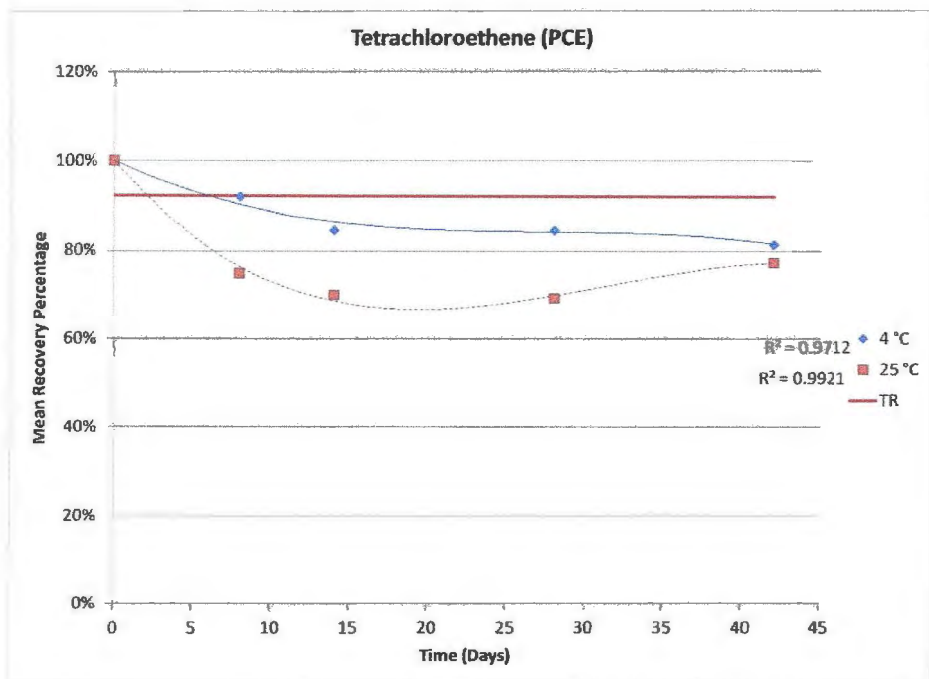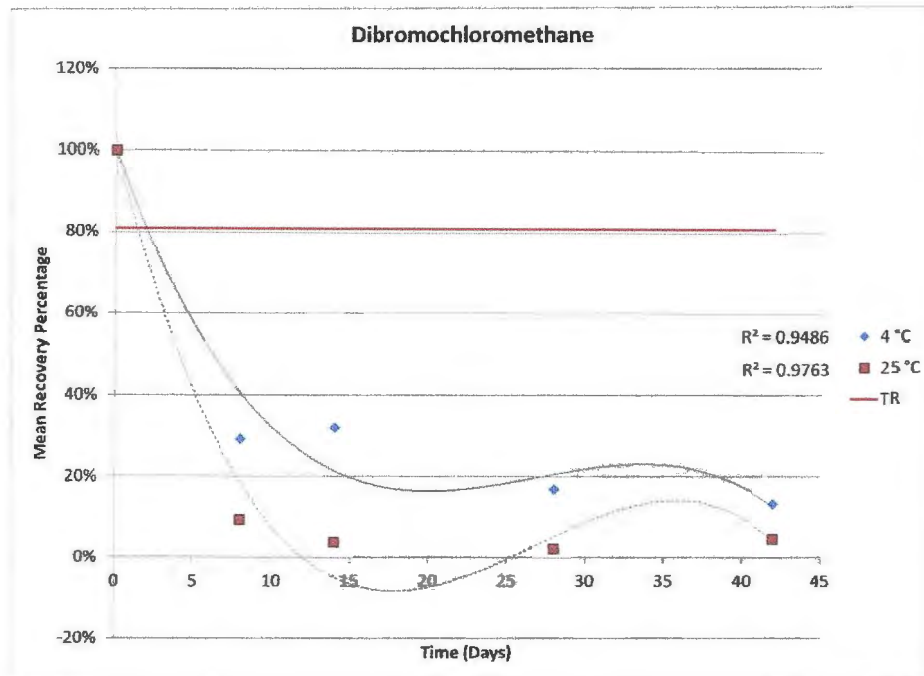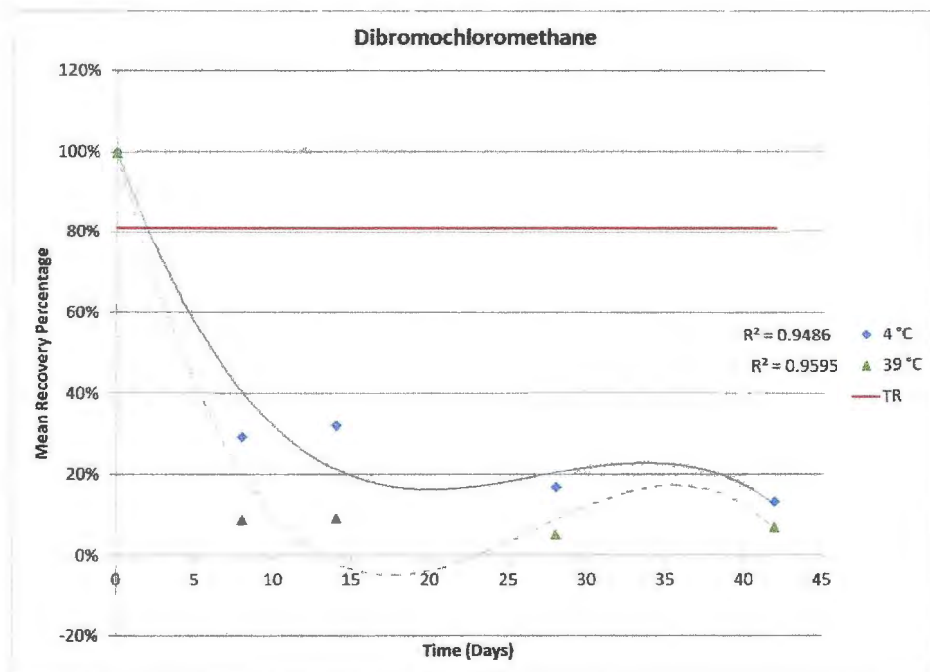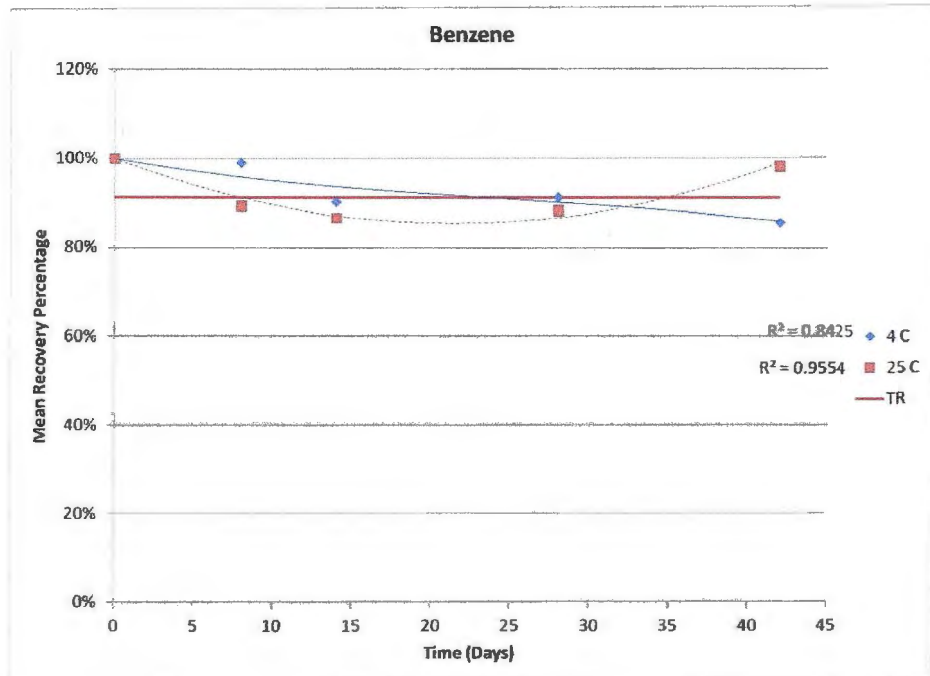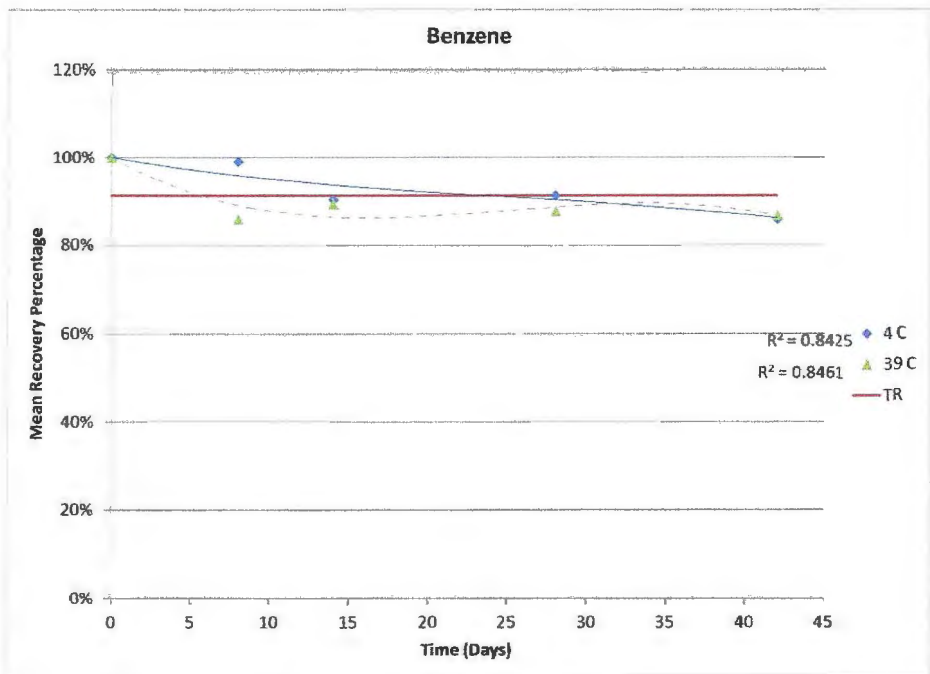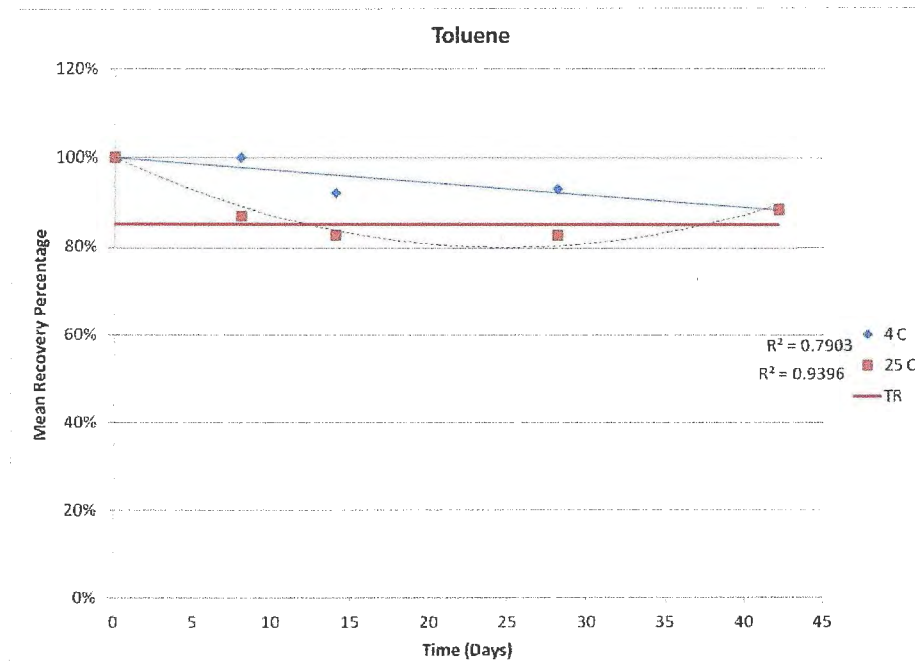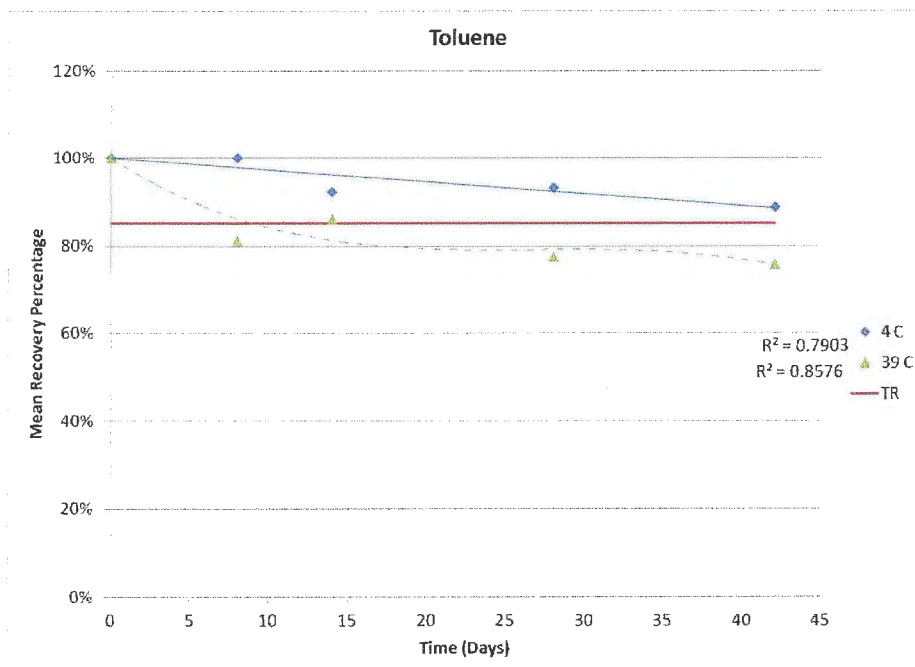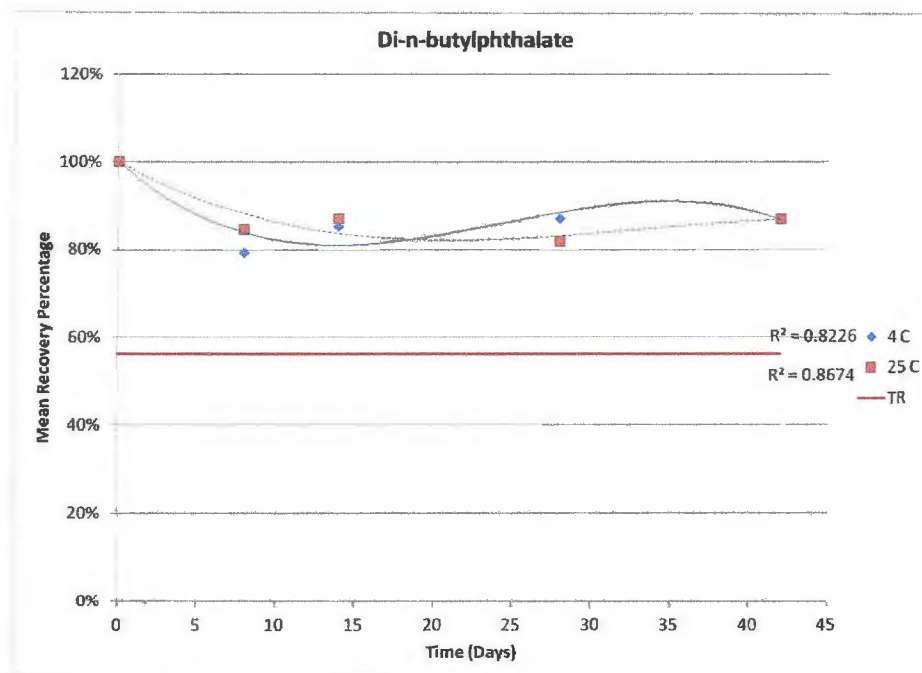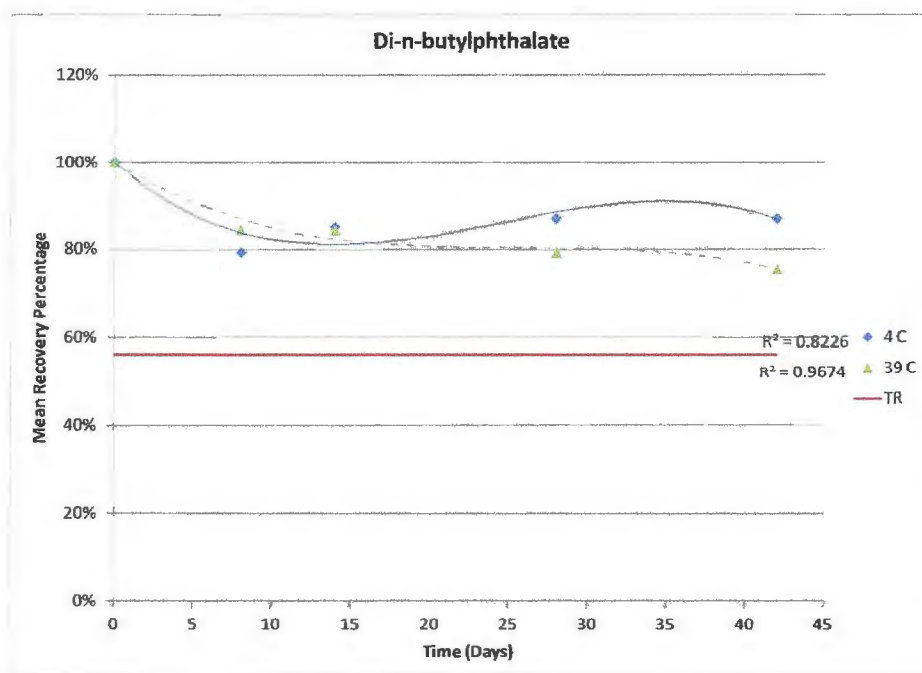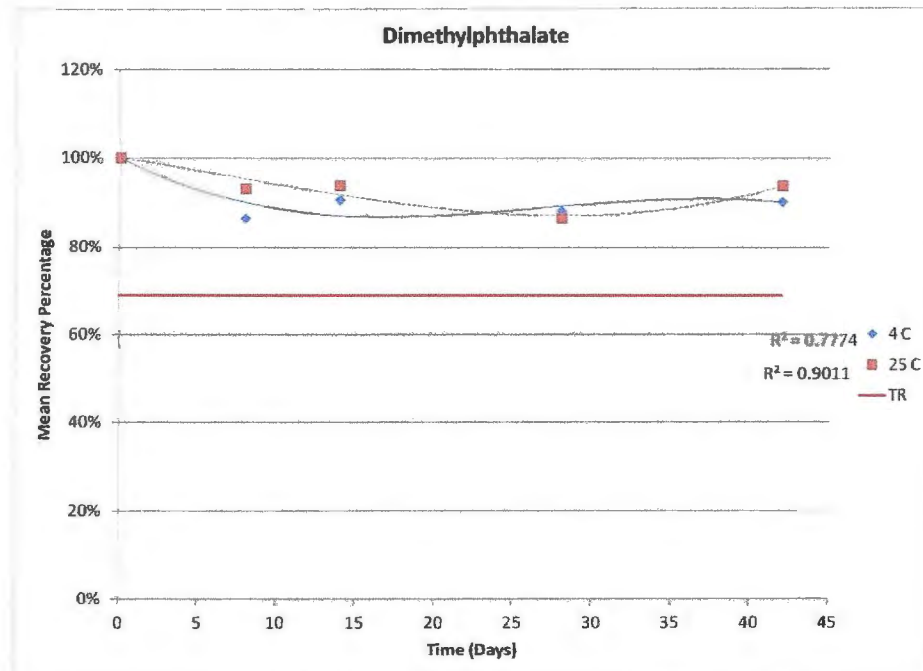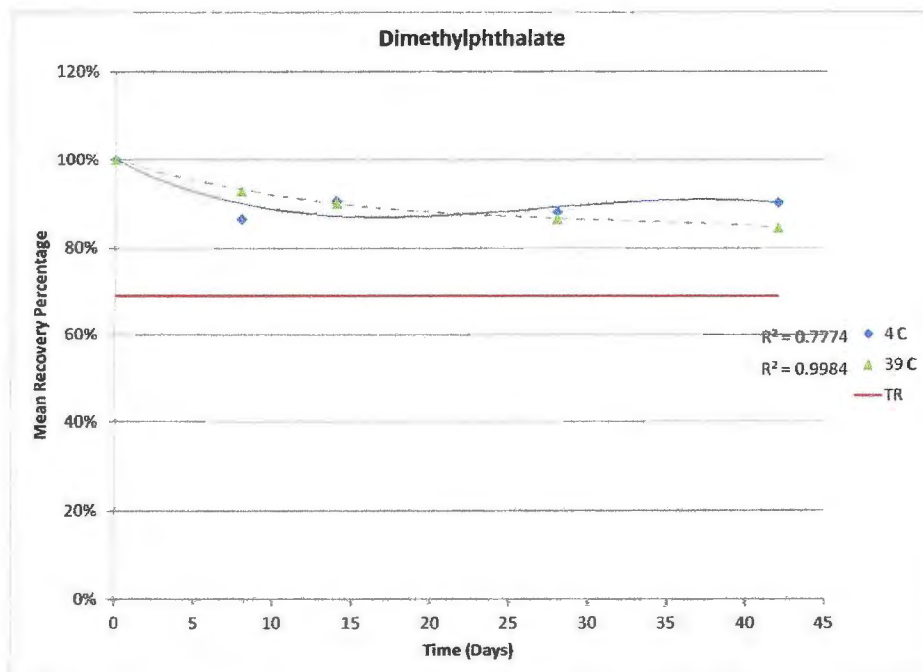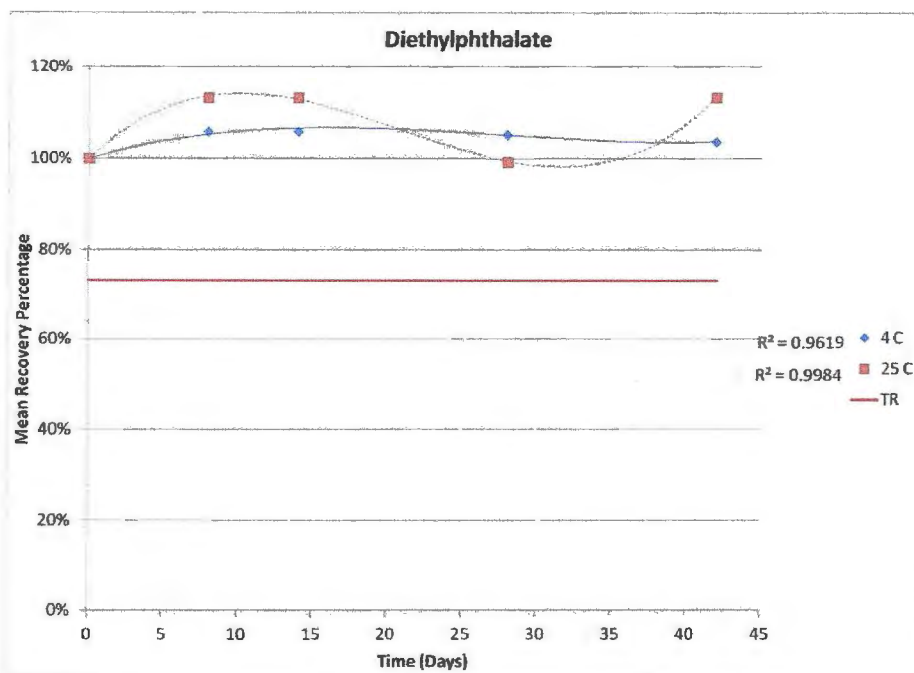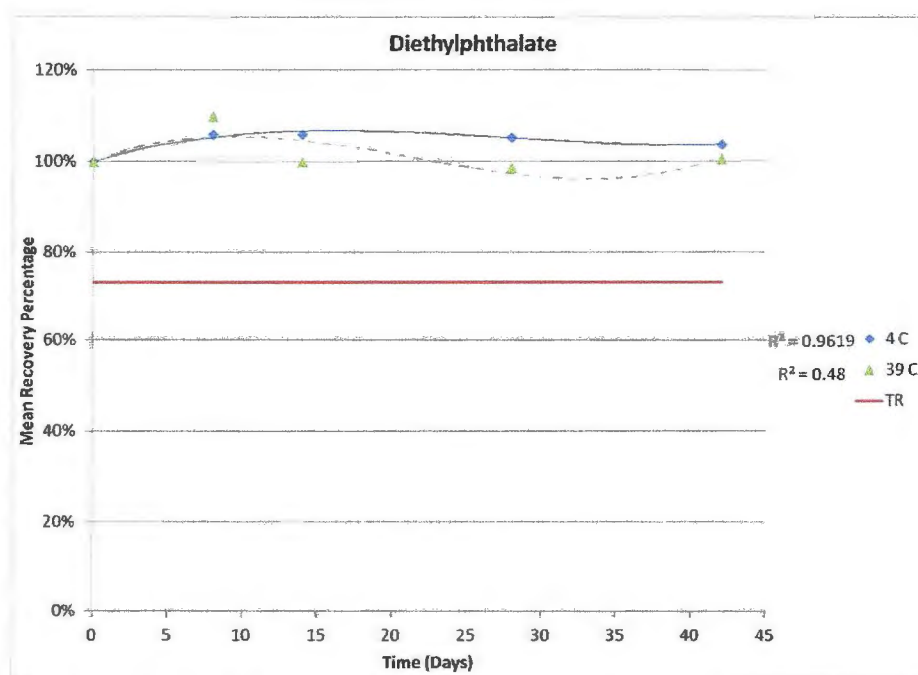
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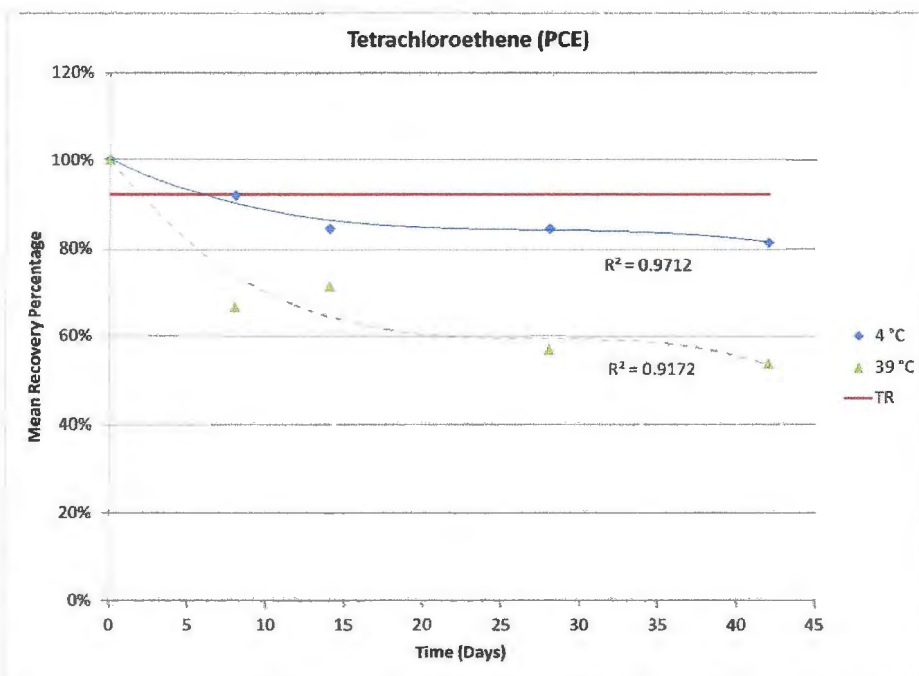
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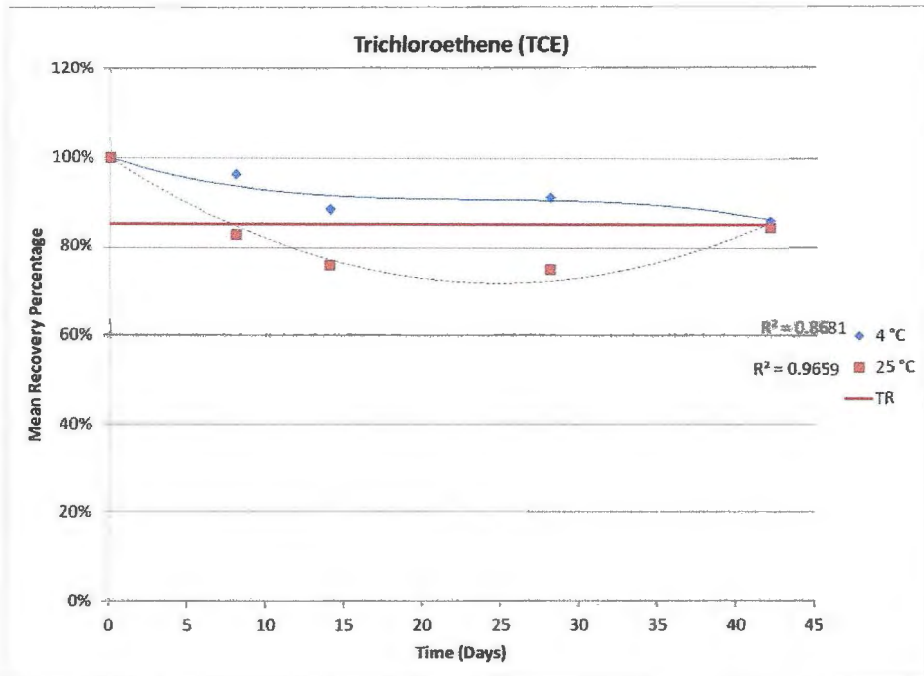
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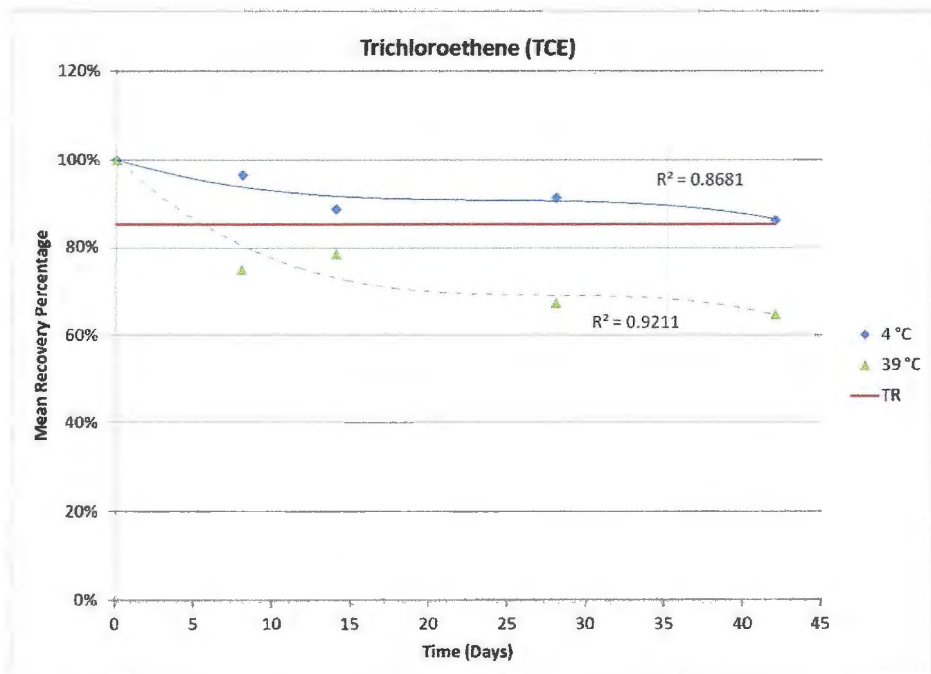
Graph #20

Graph #21

Graph #22

74
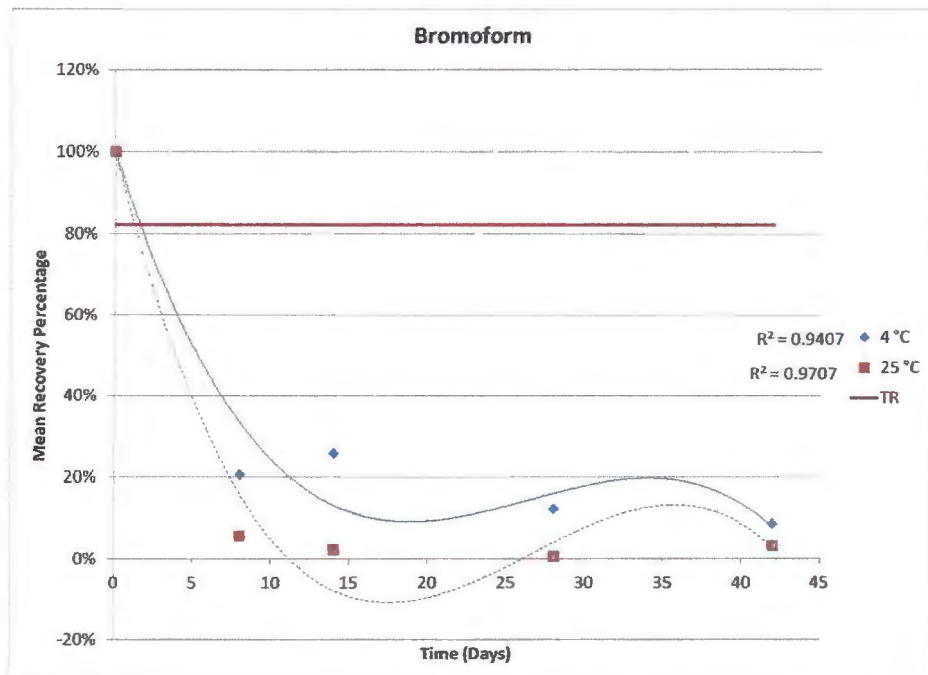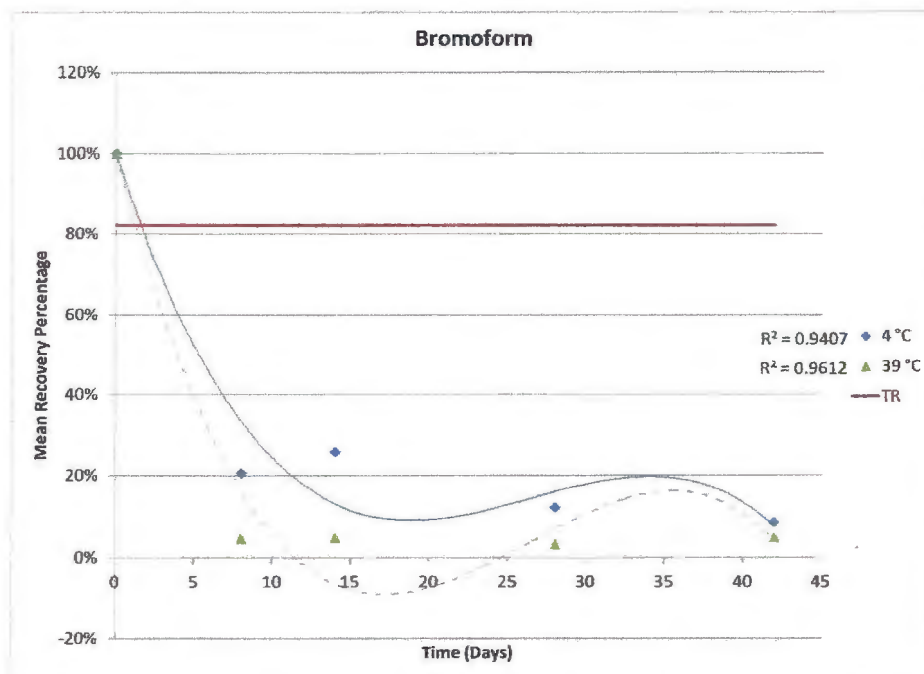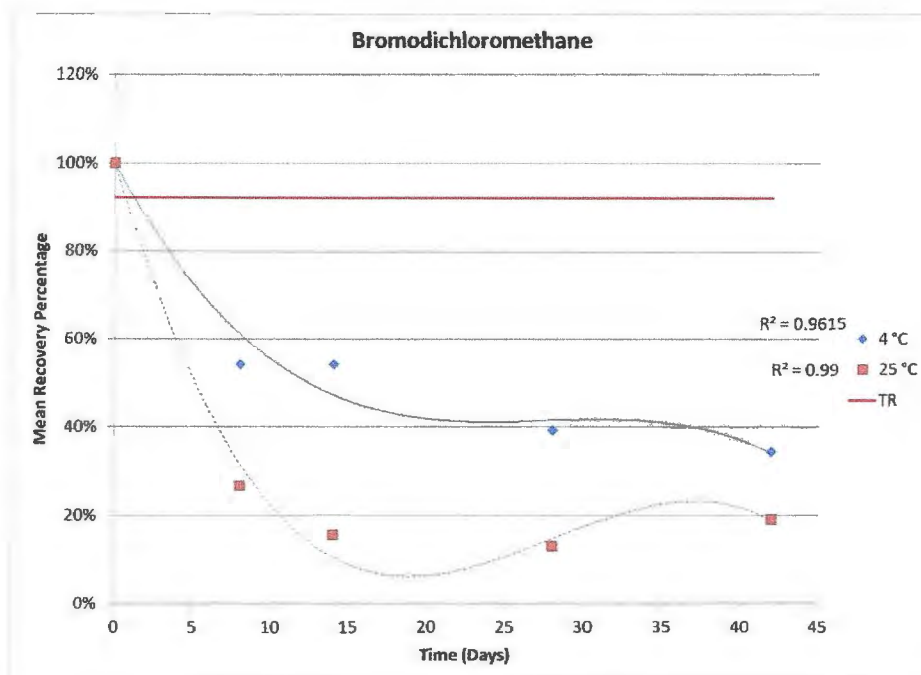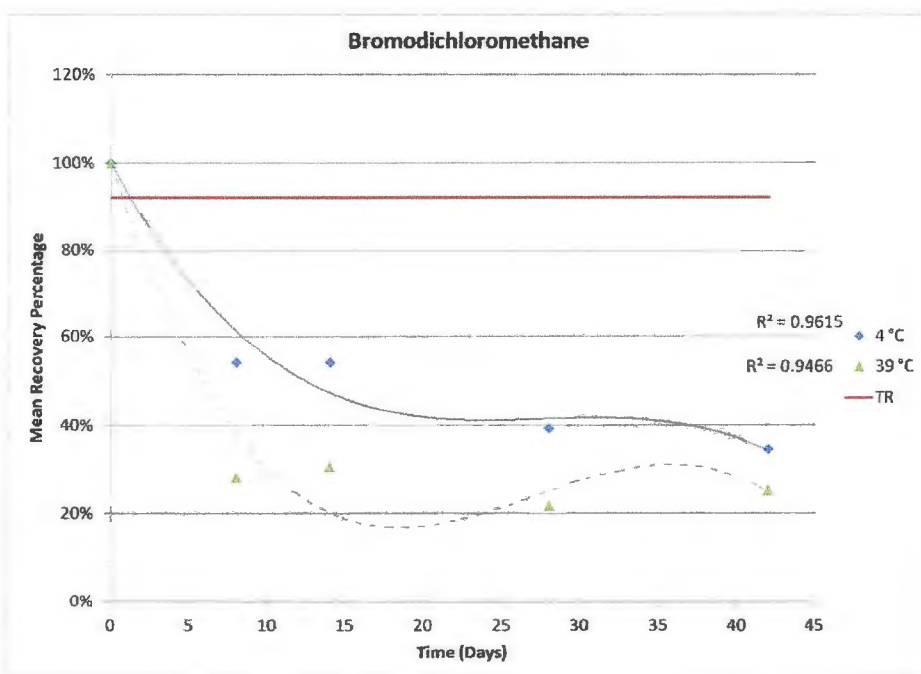
Graph #23

Graph #24

Graph #25

Graph #26

Graph #27

Graph #28

Graph #29

Graph #30

Graph #31
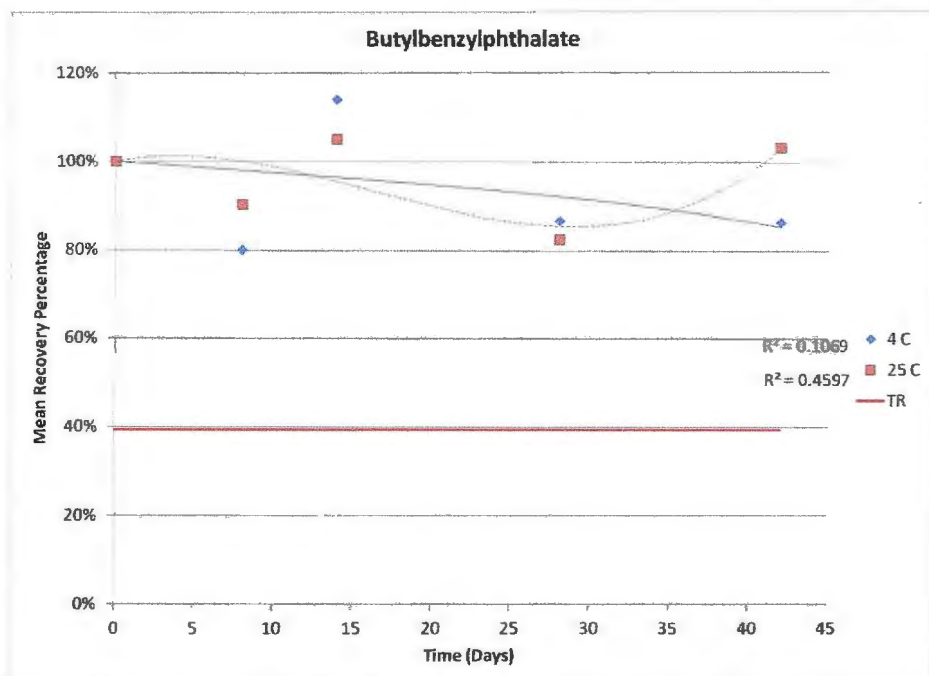

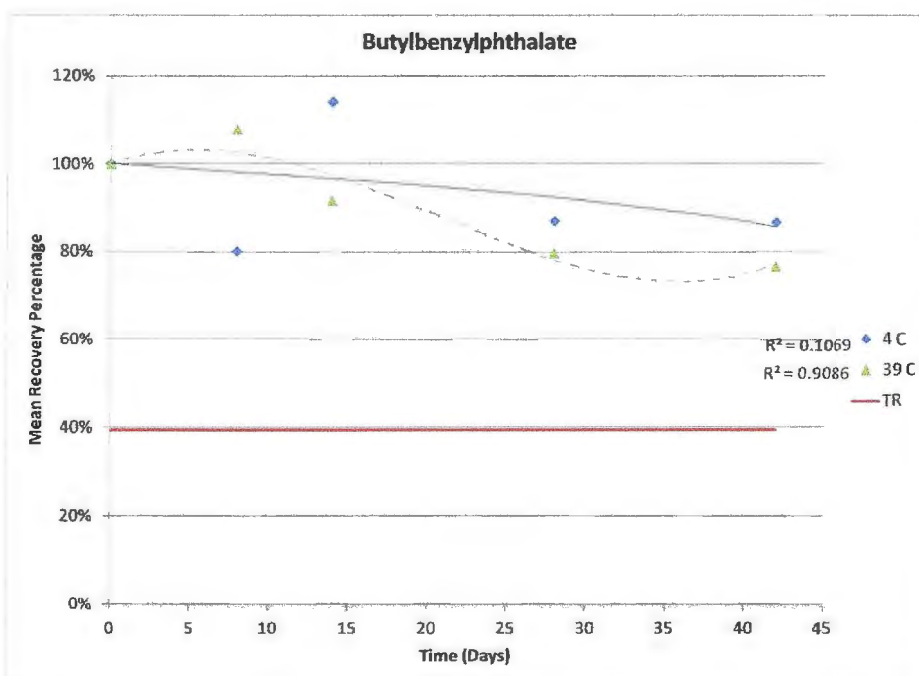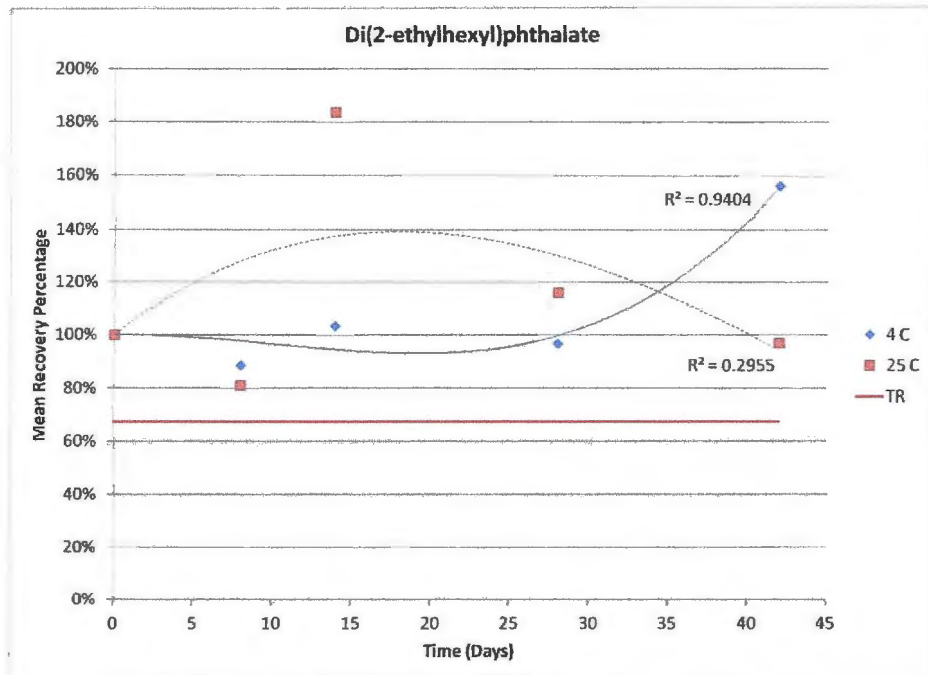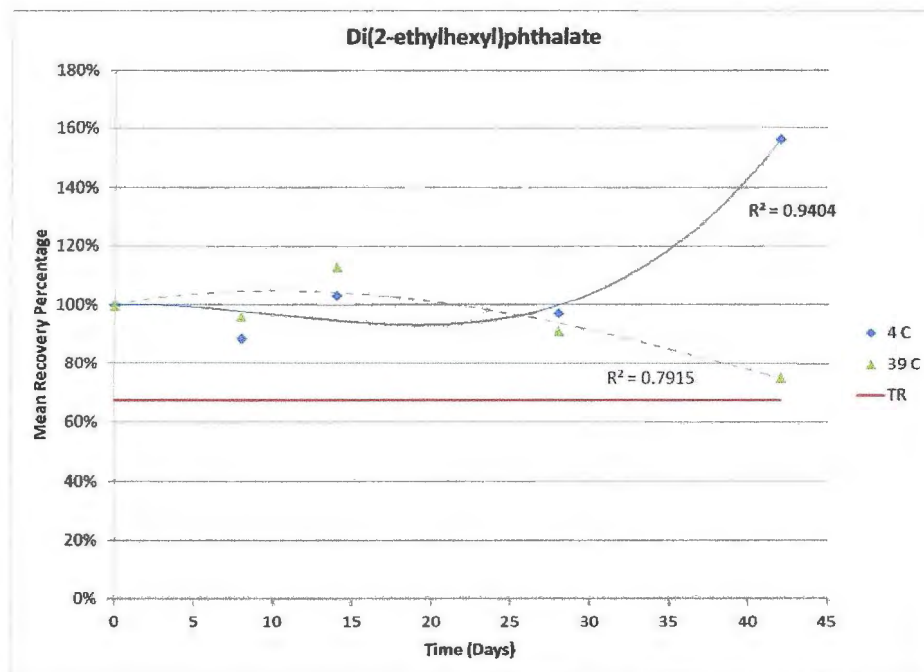Graph #32

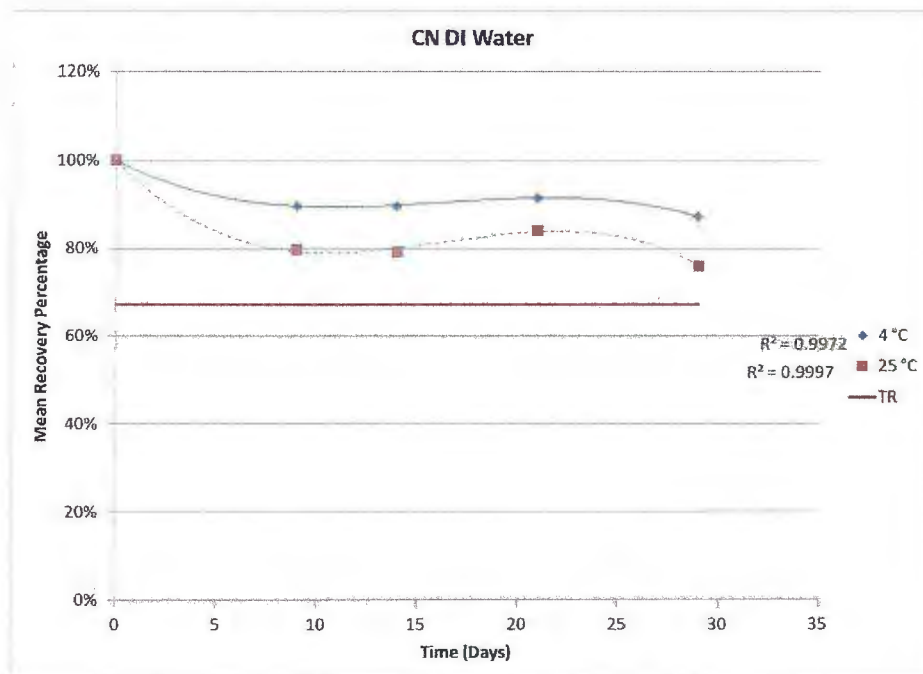Graph #33

Graph #34

Graph #35
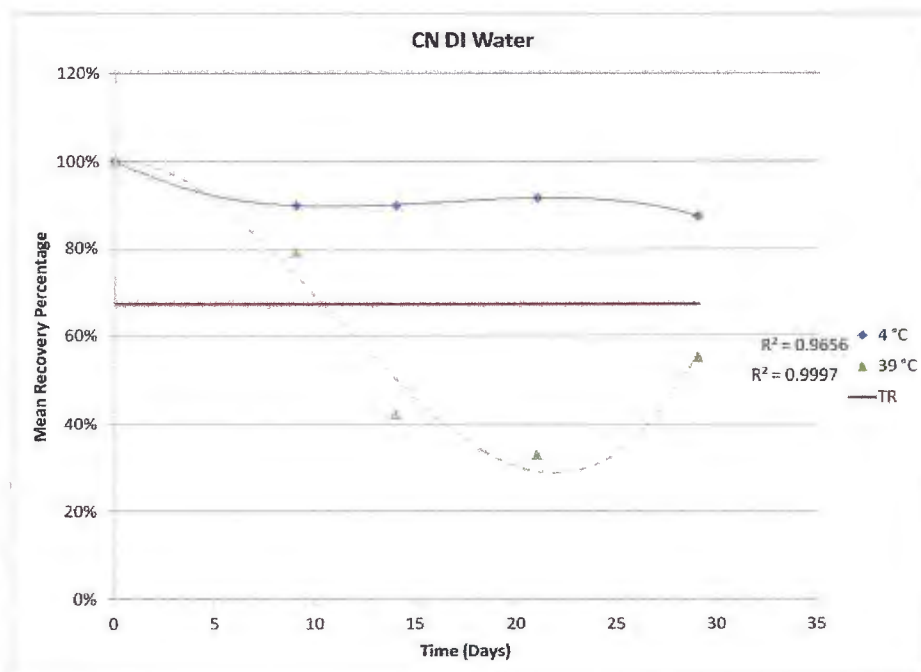

Graph #36

Graph #37

Graph #38

Graph #39

Graph #40

Graph #41

Graph #42

Cyanide

Graph #43

Graph #44

Graph #45

Graph #46

CN Preservative Study - Tap Water

Graph #47

CN Preservative Study - DI Water
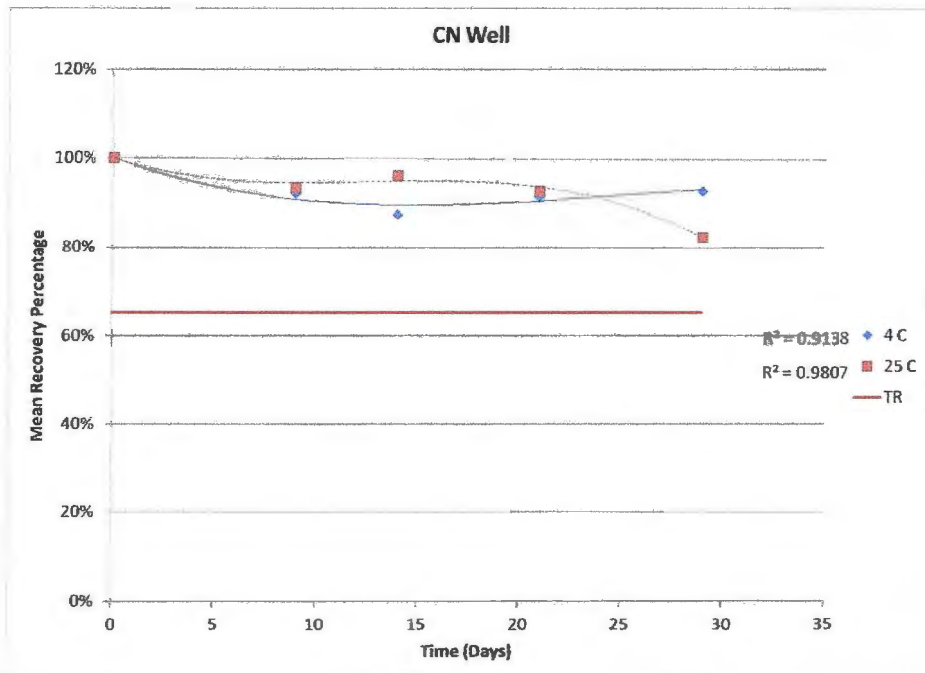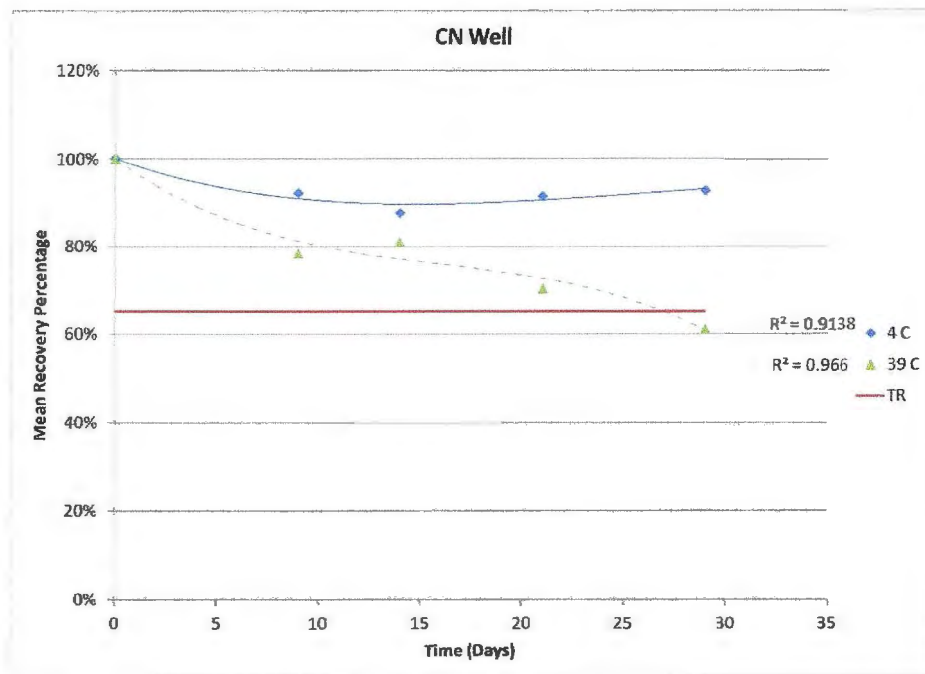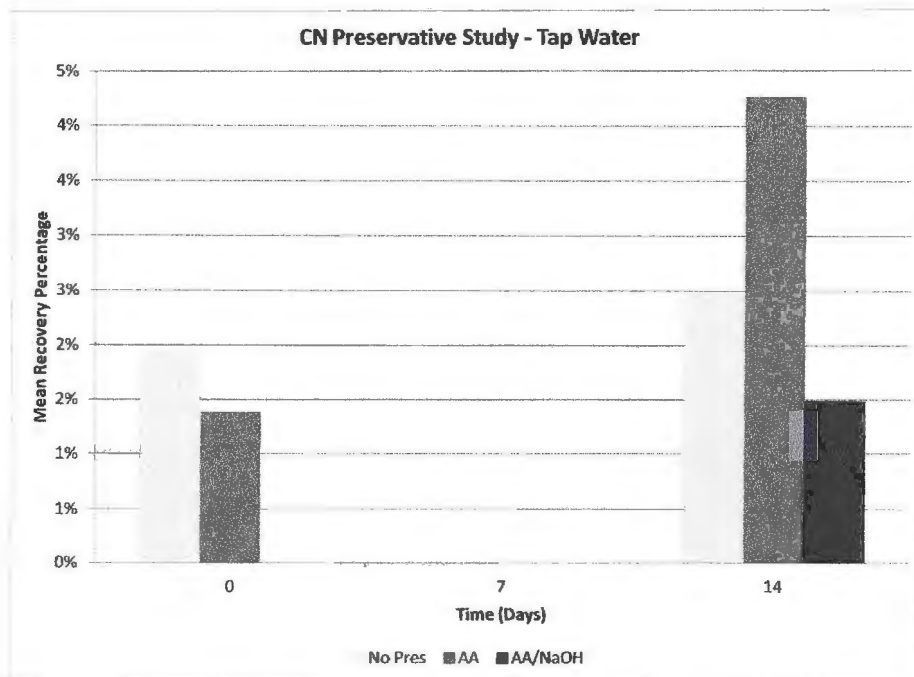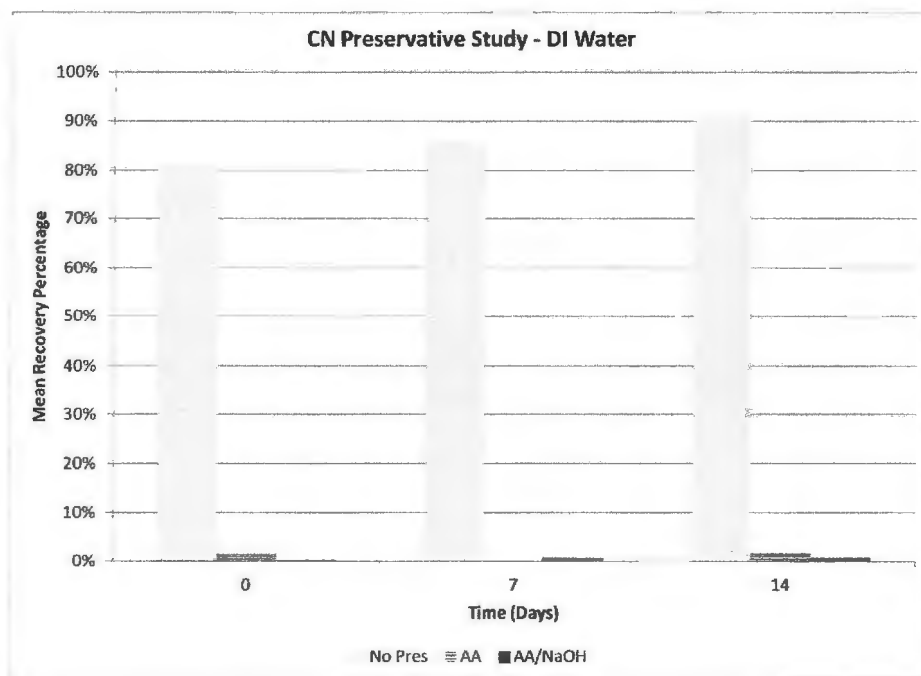
Graph #48

Appendix B – Analyte Graphing Data and Mean Percent Recovery

GRAPHING DATA

Analyte	Class	TR	TRV $n = 3$	Calculated n	Standard Deviation (s)	*RSD*
Endothall	Herbicide	142.15	357.85	69	62.45	12.49
Diquat	Herbicide	41.36	3.31	1	0.577	1.29
Paraquat	Herbicide	39.36	3.31	1	0.577	1.35
Antimony	Metals	41.27	5.73	2	1	2.13
Arsenic	Metals	41.91	8.75	4	1.53	3.01
Mercury	Metals	4.4	0.33	1	0.058	1.22
Bromodichloromethane	VOC - THM	39.02	3.31	1	0.577	1.36
Dibromochloromethane	VOC - THM	37.58	8.75	5	1.53	3.3
Bromoform	VOC - THM	30.72	6.62	5	1.15	3.09
Chloroform	VOC - THM	37.02	3.31	1	0.577	1.43
Benzene	VOC - Solvent	35.02	3.31	1	0.577	1.51
Toluene	VOC - Solvent	33.27	5.73	3	1	2.56
1,1,1-Trichloroethane	VOC - Solvent	36.36	3.31	1	0.577	1.46
Tetrachloroethene {PCE}	VOC - Solvent	38.02	3.31	1	0.577	1.4
Trichloroethene {TCE}	VOC - Solvent	33.27	5.73	3	1	2.56
Chloromethane	VOC - Solvent	36.02	3.31	1	0.577	1.47
Di-n-butylphthalate	SVOC - Phthalate	0.73	0.57	26	0.1	7.69
Butylbenzylphthalate	SVOC - Phthalate	0.43	0.66	49	0.115	10.58
Diethylphthalate	SVOC - Phthalate	0.9	0.33	10	0.058	4.68
Dimethylphthalate	SVOC - Phthalate	0.74	0.33	13	0.058	5.41
Di(2-ethylhexyl)phthalate	SVOC - Phthalate	0.83	0.41	15	0.071	5.73
Cyanide - DI Water	Ion	36.43	17.80	15	3.11	5.73
Cyanide - Well Water	Ion	32.99	17.57	17	3.07	6.06

Number of Replicates (n) = 3   Student's T (t) = 9.925
D = 15%   Tolerable Recovery Limit (*TR*) (μg/L)
Tolerable Range Variation (*TRV*)   Relative Standard Deviation (RSD)

Table B1: Data used to create stability graphs.

HERBICIDES

Endothall

		Days			
		0	8	14	28
Endothall	4 C	100%	81%	64%	80%
Endothall	25 C	100%	88%	53%	85%
Endothall	39 C	100%	101%	68%	93%

Table B2: Endothall mean percent recovery concentration study data.

Diquat / Paraquat

		Days					
		0	3	8	14	28	42
Diaquat	4C	100%	100%	101%	99%	93%	106%
Diaquat	25C	100%	79%	100%	96%	90%	107%
Diaquat	39C	100%	100%	102%	101%	93%	113%
Paraquat	4C	100%	102%	102%	102%	102%	110%
Paraquat	25C	100%	102%	102%	99%	102%	106%
Paraquat	39C	100%	101%	105%	102%	101%	109%

Table B3: Diquat / Paraquat mean percent recovery concentration study data

METALS

		Days		
		0	28	42
Metals - Antimony	4 C	100%	110%	100%
Metals - Antimony	25 C	100%	105%	101%
Metals - Antimony	39 C	100%	105%	101%
Metals - Arsenic	4 C	100%	100%	101%
Metals - Arsenic	25 C	100%	94%	99%
Metals - Arsenic	39 C	100%	94%	98%
Metals - Mercury	4 C	100%	105%	102%
Metals - Mercury	25 C	100%	108%	103%
Metals - Mercury	39 C	100%	111%	100%

Table B4: Metals mean percent recovery concentration study data

VOLATILE ORGANIC COMPOUNDS

VOC - Solvents

		Days				
		0	8	14	29	42
1,1,1-Trichloroethane	4 C	100%	102%	94%	102%	97%
1,1,1-Trichloroethane	25 C	100%	85%	83%	88%	93%
1,1,1-Trichloroethane	39 C	100%	70%	66%	51%	39%
Chloromethane	4 C	100%	103%	97%	103%	98%
Chloromethane	25 C	100%	87%	86%	89%	95%
Chloromethane	39 C	100%	78%	73%	60%	49%
Tetrachloroethene [PCE]	4 C	100%	92%	85%	85%	81%
Tetrachloroethene [PCE]	25 C	100%	75%	70%	69%	77%
Tetrachloroethene [PCE]	39 C	100%	67%	72%	57%	54%
Trichloroethene [TCE]	4 C	100%	97%	89%	91%	86%
Trichloroethene [TCE]	25 C	100%	83%	76%	75%	85%
Trichloroethene [TCE]	39 C	100%	75%	79%	68%	65%

Table B5: VOC Solvent mean percent recovery concentration study data

VOC - Trihalomethanes

		Days				
		0	8	14	29	42
Bromoform	4 C	100%	21%	26%	12%	9%
Bromoform	25 C	100%	5%	2%	0%	3%
Bromoform	39 C	100%	5%	5%	3%	5%
Chloroform	4 C	100%	102%	93%	98%	95%
Chloroform	25 C	100%	89%	87%	93%	98%
Chloroform	39 C	100%	89%	91%	93%	93%
Dibromochloromethane	4 C	100%	29%	32%	17%	13%
Dibromochloromethane	25 C	100%	9%	4%	2%	5%
Dibromochloromethane	39 C	100%	9%	10%	5%	8%
Bromodichloromethane	4 C	100%	54%	54%	39%	35%
Bromodichloromethane	25 C	100%	27%	16%	13%	19%
Bromodichloromethane	39 C	100%	28%	31%	22%	25%

Table B6: VOC THM mean percent recovery concentration study data

VOC - Benzene and Toluene

		Days				
		0	8	14	29	42
Benzene	4 C	100%	99%	90%	91%	86%
Benzene	25 C	100%	90%	87%	89%	98%
Benzene	39 C	100%	86%	90%	88%	87%
Toluene	4 C	100%	100%	92%	93%	89%
Toluene	25 C	100%	87%	83%	83%	89%
Toluene	39 C	100%	81%	86%	78%	76%

Table B7: VOC Benzene and Toluene mean percent recovery concentration study data

SEMI-VOLATILE ORGANIC COMPOUNDS

SVOC - Phthalates

		Days				
		0	8	14	28	42
Butylbenzylphthalate	4C	100%	80%	114%	87%	87%
Butylbenzylphthalate	25C	100%	90%	105%	83%	103%
Butylbenzylphthalate	39C	100%	108%	92%	80%	77%
Di(2-ethylhexyl)phthalate	4C	100%	89%	104%	97%	156%
Di(2-ethylhexyl)phthalate	25C	100%	81%	184%	116%	97%
Di(2-ethylhexyl)phthalate	39C	100%	96%	113%	91%	75%
Diethylphthalate	4C	100%	86%	86%	85%	84%
Diethylphthalate	25C	100%	92%	92%	81%	92%
Diethylphthalate	39C	100%	89%	81%	80%	82%
Dimethylphthalate	4C	100%	87%	91%	88%	90%
Dimethylphthalate	25C	100%	93%	94%	87%	94%
Dimethylphthalate	39C	100%	93%	90%	87%	85%
Di-n-butylphthalate	4C	100%	79%	85%	87%	87%
Di-n-butylphthalate	25C	100%	85%	87%	82%	87%
Di-n-butylphthalate	39C	100%	85%	85%	79%	76%

Table B8: SVOC mean percent recovery concentration study data

CYANIDE

		Days				
		0	7	14	21	31
CN - Well	4 C	100%	92%	88%	91%	93%
CN - Well	25 C	100%	93%	96%	93%	82%
CN - Well	39 C	100%	78%	81%	70%	61%
CN – DI	4 C	100%	90%	90%	91%	87%
CN – DI	25 C	100%	80%	79%	84%	76%
CN – DI	39 C	100%	79%	42%	33%	55%

Table B9: Cyanide mean percent recovery concentration study data